HISTOLOGICAL NOTES

FOR THE USE OF

MEDICAL STUDENTS

W. HORSCRAFT WATERS, M.A.

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BY

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INTRODUCTION.

In taking the class of Practical Histology at the Owens College Medical School during the Summer Sessions of 1882-1883, it has been my custom to give each student "sheets" containing a short account of the chief points to be observed in the specimens for examination. This plan has been found of great assistance, but the descriptions being of necessity extremely short, it seemed preferable to increase their length, and to obtain this result the following notes have been printed.

These notes are by no means intended as a full description of the histological structure of the tissues, neither are they given for the instruction of the student in the various methods of section-cutting, injection, etc.; but simply to aid him in his examination of specimens.

The specimens were given to the student ready for mounting, since time would not allow him to stain and clear each section for himself; but it is desirable that this course should be followed now and again.

The various staining, clearing, and mounting reagents are those in general use, but a brief account of those hardening processes, etc., which gave very good specimens, has been included.

One feels there cannot be too closely impressed upon a student the necessity of introducing method into his examination and description of specimens, and of making sketches to assist himself and his instructor.

In the description of tissue preparation the same numbers and arrangements have been followed as those used in describing the structure of the specimens.

I am much indebted to Prof. Gamgee and Prof. Marshall for many kind suggestions, which have assisted me greatly in preparing these notes.

HISTOLOGICAL NOTES.

I.—MOUNTING, etc.

The microscope having been placed in a convenient position, and with care that the stand is firm and perfectly steady, the curved mirror is to be adjusted so as to throw a beam of light through the stage-aperture and along the central axis of the tube. Direct sunlight is not to be used for illumination; that thrown from some bright cloud is strongly recommended.

The eye-pieces and object-glasses, as well as the mirror, are to be kept *perfectly* clean and free from dust by means of a soft piece of wash-leather, which should on no account be used for any other purpose.

Specimens must *invariably* be examined *first with the low power and then with the high power*, using with the former a medium sized diaphragm, and a somewhat smaller one with the latter. Before placing or removing specimens, the objective is always to be withdrawn some distance from the stage by sliding the telescope-tube upwards with a gentle twist.

When placed, the object may be brought to a focus by gently passing the telescope-tube downwards till the specimen is in view (*coarse adjustment*), and then by slightly turning the screw at the top of the stand, until a clear and distinct image is obtained (*fine adjustment*). Care is to be taken that this fine-adjustment screw is not turned too much to the left so as to rise far out from the stand, nor too much to the right and so become fixed.

It is best to accustom oneself to working with both the left and the right eye, at the same time keeping open the one not directly in use. By this means the eyes become less tired, and with a little practice the whole attention

will be thrown upon the object under examination. It is also of great assistance in making sketches of the preparations.

I. Cell.

Yeast. Have a perfectly clean slide and coverslip. Place the former upon some dark surface, dip the end of a clean glass rod into a solution containing yeast, and then touch the centre of the slide, leaving a small quantity of the solution upon it. Holding the edges of the coverslip between the thumb and forefinger of the left hand, and having a mounting needle in the right one, rest the left edge of the coverslip upon the slide slightly to the left of the yeast drop, and the right edge upon the needle; then gently lower the latter till the slip touches the yeast and covers it. By this means a specimen may be mounted with less chance of having *air-bubbles* present.

At times the body to be mounted is placed upon the coverslip, which is then turned over and lowered upon the slide in a similar manner.

Be careful in choosing the quantity of solution. If a drop of insufficient size be taken, air-bubbles or air-spaces will be present; while if too large a one be used it will spread over the slide and perhaps the coverslip, when it cannot be examined. The latter is of great importance, and a remount should invariably be made, as any matter upon the upper surface of a coverslip renders the specimen quite useless.

The yeast having been satisfactorily "put up," should first be examined with the low power (L.P.). The specimen appears as a fluid in which are floating *cells* of variable size, some single and others collected in small groups.

Examine under the high power (H.P.). The cells consist of a *cell-wall*, seen by its double outline, surrounding the *cells-contents*, a mass of granular substance, *protoplasm*. This protoplasm of the larger cells may have one or more clear spaces, *vacuoles*, which are not to be mistaken for nuclei, being areas of less dense protoplasm.

Sketches should now be taken of several cells, both large and small. Placing the sketch book to the right side of the microscope and level with the stage, draw a

faint outline of the cell with the H. pencil, and then carefully finish with the H.B.; making the general protoplasm somewhat darker than the vacuoles, and the granules still darker.

Sketches of specimens should *always* be made, and it is best to draw them of the apparent size, or some multiple of the apparent size; while a note may be placed at the side somewhat after this manner. If under the low power, L.P.; if under the high power, H.P. Under the low power and of the apparent size, L.P. × 1; or, if under the low power and three times the apparent size, L.P. × 3, etc.

II. Application of Reagents.

In studying the microscopic structure of various bodies it is necessary to add different reagents and staining fluids. Frequently it is best to put up the object and then add the fluid, which may be done :—

1. By the use of a glass rod and blotting paper; or
2. By using the rod and a fine capillary tube.

As an example study the staining power of a magenta solution upon yeast.

Mount a specimen of yeast as before and focus under the H.P. Now dip a rod into the magenta solution, place a drop some slight distance to the left of the specimen, and, with a needle, lead up a thin stream of the fluid to the coverslip. On the opposite side of the latter place one corner of a small triangular piece of blotting paper; as soon as this touches the yeast solution a stream will cross the field of the microscope, the magenta flow in, and the cells become stained. The cell-walls will receive no colour, but the protoplasm will stain well, the vacuoles less deeply—a characteristic in which they differ from nuclei.

In place of the blotting paper a fine capillary tube may be used, with the same results.

III. Testing.

By using reagents in the above manner, many substances may be detected under the microscope by characteristic reactions. As examples of this the reactions of starch and of cellulose may be used.

1. *Starch.* Mount a few granules of starch, by grinding some in cold water and covering a drop upon a slide. Examine under H.P. Pass in a dilute solution of iodine, and the granules turn *blue*, a characteristic reaction.
2. *Cellulose.* Mount some scrapings from the cut surface of a potato. Examine under the H.P., and run in a little iodine solution; the starch granules present turn blue, and the walls of the cell-spaces containing them take a yellow tint. If in a similar manner dilute hydric sulphate be now added, this yellow tint will change to a *blue* one, indicating *cellulose.* The same reaction is given by cotton fibres, or the walls of the yeast cells.

IV. Foreign Bodies, etc.

In making microscopic preparations, certain bodies from time to time become present which it is necessary to carefully distinguish from the tissues under examination. The more common of these should therefore be placed under the microscope, and sketches made of them, in order that their characteristics may be known.

1. *Brownian movement.* This vibratory movement should be carefully distinguished from any vital one; it is shown by some specimens of living matter, but also by non-living matter, and is essentially a physical phenomenon.

   (*a*) *Gamboge.* Grind some gamboge in water, and examine a drop under H.P. Various currents may be detected, but also this vibratory, Brownian movement of the small granules.

   (*b*) *Bacteria.* Mount a specimen from a watery extract of boiled meat, which has been allowed to stand a few days. Some very fine bacteria will show a similar motion, which lasts after their death, brought about by the addition of iodine or boiling the solution.
2. *Cotton fibres.* Mount in water a few fibres of cotton wool, cover and examine under L.P. and H.P. They appear as long, flat threads with a distinct twist, and turn blue on the addition of iodine and hydric sulphate.
3. *Flax fibres.* Examine in a similar manner some flax fibres; these are long and flat, but unlike cotton ones do not show any twist.

4. *Air-bubbles.* Shake a solution of gum in a test-tube till a froth appears, then mount a drop of the under fluid, and examine under L.P. and H.P.

By transmitted light the air-bubbles appear as a bright centre, surrounded by several dark rings of various depths; by alteration of the focus these rings will change.

With reflected light (produced by shutting off that thrown from the mirror) a reversion takes place, the centre becoming dark and the periphery light. Sketch.

5. *Fat globules.* Mount a small drop of milk and compare the fat globules with (4). Notice their sharp definition and variation of size; pass in a little sodium hydrate and they immediately run together.

V. To Prevent Drying.

In examining specimens of tissue for some length of time it is often necessary to prevent evaporation of the mounting medium at the edge of the coverslip, which tends to cause an alteration in their appearance.

1. **With fresh tissues**, which are usually examined in serum or normal saline (·75 % solution of common salt) use may be made of oil, paraffin or cocoa butter.

Oil: best salad oil. After covering carefully to prevent any fluid coming beyond the coverslip, a thin film of oil is to be immediately painted round and slightly overlapping the latter. For this purpose a fine camel-hair pencil is used, and is best kept with its holder piercing the cork of the oil bottle, that it may be free from dust, etc.

Paraffin requires to be melted in a capsule before application. A good preparation may be made of 15 cc. of liquid paraffin and 15 grms.: of solid paraffin, kept for about an hour between 40° C. and 50° C. It melts at about 38° C., and is useful in warm-stage experiments for studying the amœboid movements of white blood corpuscles; the commencement of melting being an indication of the limit of temperature. The same precautions must be taken as in the use of oil.

Cocoa butter. Its use and application are similar to those of paraffin.

2. **Sections, etc.**, which have been mounted in glycerine or

any media liable to evaporation, require the coverslip to be surrounded and slightly overlapped by some cementing fluid, which will gradually dry and form a firm ring. For this purpose white varnish, brown cement, etc., are good.

The cement is best applied by *centering* the coverslip upon a *turn-table* (that is, arranging the border of the coverslip concentrically with the circles on the brass plate), fixing the slide down with the clips, and while rapidly turning to gently bring a camel-hair pencil holding the cement, down to the edge of the slip. This slip *must* be carefully centered and the slide *firmly* clamped, otherwise the rapid rotation of the table will throw off the specimen. To square coverslips the cement may be applied by gentle painting.

VI. Mounting.

Preparations of tissues which it is desired to keep, are usually mounted in Glycerine or in solutions of Canada Balsam or Dammar. The solvent of the last two bodies evaporates and leaves a clear hard ring, so that no cement is required as in the case of Glycerine.

The mounting medium should be quite mixable with that from which the specimen is taken: it being required in some cases to use several fluids to obtain this result.

Tissues and sections, both stained or unstained, are as a rule kept in *spirit*, so the following order should be followed in mounting:—

For Glycerine.	For C. Balsam (two ways).	
1. Spirit.	1. Spirit.	1. Spirit.
2. Water.	2. Absolute Alcohol.	2. Absolute Alcohol.
3. Glycerine.	3. Turpentine and Kreosote (4:1).	3. Oil of Cloves.
	4. C. Balsam.	4. C. Balsam.

Glycerine. Pure glycerine may be used or a mixture of equal parts of glycerine and water, which is somewhat preferable. For quick mounting, for specimens which are unstained or hyaline in character, and for those which require teasing, it is better than C. Balsam.

A section-lifter is required in handling specimens; and great care should be taken in removing them from spirit

to water: the surface currents causing rapid movements which may lead to the breaking-up of a section. The time during which they should remain in water before removal to glycerine depends upon their size and density, from five to ten minutes being generally sufficient.

Canada Balsam or Dammar. These are soluble in chloroform, turpentine or benzole, being used for the less transparent and for the stained tissues, rendering specimens which are mounted in them very clear.

The media required in mounting from spirit are to be applied in the order given above; the specimen being changed from watch-glasses containing them by a section lifter, after removing any excess of each fluid clinging to the specimen by a piece of blotting paper.

The specimen may remain on the slide, and the media applied by pipettes and removed by blotting paper after their action.

Five to ten minutes is usually sufficient time for a section to remain in each fluid.

Take great care that all needles, seekers, section-lifters, etc., are *quite clean* from one fluid before being placed in another; and on no account must the rod or brush of one reagent-bottle be allowed to touch another reagent.

II.—BLOOD.

I. Frog's Blood.

1. Upon a perfectly clean slide place a very small drop of blood from a frog just killed, cover immediately with a clean slip, and quickly surround with oil. Examine first with L.P. and then with H.P.; notice the many *red* corpuscles and few *white* ones floating in the *plasma.*

Red corpuscles; number: very great. Size: . Colour: when seen separately they have a yellow tint, but when collected this deepens to a red one. Shape: flat discs, seen from above they appear elliptical, but seen edgeways they are very thin, with a gradual swelling in the

middle portion—the position of the *nucleus*; after a time, particularly in those seen flat, this nucleus becomes more evident and occupies about one third of the area.

White corpuscles; number: far less than the red ones. Size: slightly smaller than the red. Shape: if examined quickly it is indefinite, and changes from time to time, *amœboid movements*, but on keeping they become round. In appearance they are white with a number of fine *granules*. Sketch at short intervals a particular corpuscle, to detect any amœboid movements.

The action of various reagents must now be studied.

2. **Water.** Mount a fresh specimen, but do not surround with oil as a reagent has to be added. Run in a little water, by the use of a glass rod and blotting paper as described. The corpuscles swell out to a globular form, and the colouring matter of the red ones, *hæmoglobin*, diffuses into the surrounding fluid, leaving the remaining portion, *stroma*, indistinctly seen.
3. **Normal Saline** (·75 % solution of sodium chloride). To a fresh specimen add normal saline in a similar manner, no change will take place in either the red or white corpuscles. For this reason the other reagents to be used should be diluted or dissolved in normal saline, and not in water.
4. **Acetic Acid 1 %.** Add to a fresh specimen as with the above.

   Red corpuscles: swell; lose their colour, the outline becoming indistinct, while an oval shaped *nucleus* appears at the centre.

   White corpuscles: become clear with a few granules; a *nucleus* appears, but not, as a rule, in the centre: at times more than one may be present.
5. **Dilute Alcohol** (1 part alcohol, 2 of normal saline). This will cause the red corpuscles to swell, and their nuclei to appear.
6. **Syrup:** to be made as a weak solution of sugar. By its action the red corpuscles shrink and assume an irregular, indefinite shape.
7. **Magenta.** By the addition of this reagent both corpuscles stain, but their nuclei do so far more deeply, and become very distinct (page 56).

8. Boracic Acid 2 °/₀. This acts upon the red corpuscles, causing the colouring matter to separate from the stroma, and collect round the nucleus.

II. Bird's Blood.

Remove the head of a pigeon and allow the blood to flow into a very small vessel, such as a porcelain crucible. Whip sharply with a few horse-hairs (or small glass-rod) to remove the fibrin, and keep covered with a piece of waxed glass well pressed upon the edges of the crucible. It may be diluted with ·75 % salt solution, and will keep a short time for specimens. If sufficient blood be not obtained, the heart may be cut and more collected.

1. Mount a small drop, as with frog's blood, surround the coverslip with oil, and examine with L.P. and H.P.

Red corpuscles; numerous. Size: much smaller than those of the frog. Shape: elliptical discs, but very pointed at the ends; possess a *nucleus*.

White corpuscles, few in number. Shape: very irregular, but tend to become round while mounting, the warm-stage being required to demonstrate their amœboid shape and movements. They are granular, and their *nuclei* may be demonstrated by reagents.

2. **Acetic Acid, 1 °/₀.**
3. **Syrup.**
4. **Magenta.**
5. **Boracic Acid, 2 °/₀.**

Apply in the same way as with frog's blood, and similar results will follow.

III. Human Blood.

Specimens may be obtained by pricking the top of the finger with a clean needle.

1. Mount a very small drop, quickly surround with oil, and examine under L.P. and H.P.

Red corpuscles, very numerous. Size: smaller than those of the frog. Colour: yellow or yellowish-red if seen separately, but a distinct red when grouped together. Shape: circular biconcave discs; this concavity may be well detected from the dumb-bell appearance of those seen edgeways, and it is the cause of those seen flat having a dark edge with a bright centre or a bright edge with a dark centre, according to the focus of the micro-

scope. After a short time they collect in rows with their flat sides together, forming lines or *rouleaux.*

White corpuscles, few in number. Size: just slightly larger than the red. Shape: irregular, but, like those of the bird, they become globular. The cell substance is *granular* and *nucleated.*

2. **Acetic Acid, 1%.** Causes a similar effect to that in the frog, but *no nucleus* appears in the *red* corpuscle. The white are nucleated.
3. **Syrup.**
4. **Normal Saline.** } Effects, the same as in frog and bird.
5. **Drying.** Mount a specimen, do not surround with oil, and examine under H.P. The red corpuscles will gradually shrink and become *crenate* at their edges, from evaporation taking place at the border of the coverslip and a consequent increase in the density of the fluid.
6. **Tannic Acid, 1%.** Examine under H.P. This breaks the physiological connection of the hœmoglobin and stroma of the red corpuscle, and the former collects in a small knob at the border of the latter.

Demonstrations.

1. The amœboid movements of white corpuscles in a specimen of frog's blood placed on a warm stage.
2. Fibrin filaments in coagulated blood.
3. Hœmoglobin crystals.

III.—EPITHELIUM.

I. Squamous.

1. Having previously rinsed the mouth with water, gently scrape the inside of the cheek with the handle of a clean scalpel. Mount the specimen free from air-bubbles, and examine with L.P. and H.P.

(*a*) Notice the large *squamous* epithelial cells, frequently collected in small groups. They are flat, with irregular, curling edges; clear, transparent, and possess a *nucleus*. (Some small granular cells may here and there be seen—the salivary corpuscles.)

(*b*) Run in a little 1 % acetic acid to the above, and the *nuclei* of the epithelial cells will appear more distinctly.

(*c*) To a fresh specimen add a small quantity of magenta solution, and the nuclei will stain very well.

2. **Cast skin of the newt.** Stain with logwood and mount in C. Balsam, after passing through the various clearing fluids as previously described (page 6). Examine under L.P. and H.P.

A layer of *polygonal* shaped *epithelial cells* will be seen, placed edge to edge, and showing at their centres well stained, granular *nuclei*. Here and there stomata appear.

II. Columnar.

1. **Intestine** of rabbit, frog, etc. Gently brush the surface of the small intestine, treated for 24 hours with Ranvier's alcohol and stained in picro-carmine; carefully break up these scrapings with needles in a little glycerine, and examine with H.P.

The *columnar* epithelial cells, single or grouped, have a substance of *granular protoplasm* with a clear *hyaline border* at the top, and possess a distinct *nucleus*.

In places *goblet cells* may be seen, the body containing but little protoplasm, being chiefly formed of mucin, which does not stain; the nucleus is small, and pressed towards the border of the cell.

III. Ciliated.

1. **Trachea** of rat or rabbit. Prepare and mount as described for the intestine: examine under H.P.

The cells are *columnar* and *nucleated* as above, but in addition, a layer of distinct *cilia* will be seen on the *hyaline border* at the top of the cell.

2. **Ciliary movement.** In a frog just killed, gently scrape with a clean scalpel the roof of the mouth near the

eye-ball: mount the scraping in normal saline, cover very gently, and use the H.P.

Ciliated epithelial cells will be seen, the cilia being in *rapid motion*, and frequently causing movement of the cells or of any fine granules near them. After a time this becomes less vigorous, and can be more clearly seen; it finally ceases as the cells die.

IV. Pigmented.

1. **Pigmented epithelium of the retina.** Carefully remove a piece of this layer from a young eye (prepared) and mount, unstained, in Canada Balsam. If the retina be still attached be sure to mount with the pigment layer uppermost. Use L.P., then H.P.

The cells are *hexagonal* in shape, from mutual pressure; they contain the *pigment* in fine granules, which are not so dense round the *nuclei*, these therefore appear in clear spaces. If any cells are observed edgeways, the outer surface is seen straight and clear; the inner one irregular, and containing the pigment granules.

V. Stratified.

1. **Bladder.** Tease in glycerine the epithelium removed from a dog's bladder treated with Ranvier's alcohol, and stained with picro-carmine. Use L.P. and H.P.

Notice the *various shaped, nucleated cells*, passing from the superficial *flattened* to the lower, more *columnar* variety. The former show surface markings, due to their pressure on the lower ones.

2. **Cornea.** Take a section of the cornea stained in logwood and mount in C. Balsam.

Under L.P. notice the deeply stained, broad line on the surface, and examine this under HP. This is the outer layer, and consists of a series of *stratified* epithelial cells, passing from the *columnar* shaped ones next the general corneal substance, to the superficial *squamous* variety: each cell has a *nucleus*.

IV.—CONNECTIVE TISSUES.

Cartilage and Bone will be considered in separate divisions. The three chief factors of connective tissue are (1) yellow, elastic fibres, (2) white fibres, and (3) connective tissue corpuscles; as far as possible these should be examined separately, and then collectively as forming the ordinary connective or areolar tissue.

I. Yellow, Elastic Tissue.

1. Ligamentum nuchæ, or lig: subflava. Tease very carefully a piece of the lig: nuchæ of an ox. This *teasing* should be done by firmly fixing on the slide (with a needle held in the left hand) one end of the piece of tissue, and continually working another needle (held in the right hand) from left to right along the tissue; by this means the tissue will be broken up into a number of fine filaments. The fibres may be separated by working the needles in a lateral direction to that of the fibres. Now cover with a slip by resting its left edge upon the slide and lowering it with the right needle: by this means a slight current will flow, and float apart the finely divided fibres. Use L.P. and H.P.

   Note the thin *yellow*, slightly anastomosing fibres, *curling* at their free ends. They possess a very *distinct* and *clear outline.*

   Run in some 5 % acetic acid, the fibres remain quite *unaffected.* (N.B.—There may be a small amount of white fibrous tissue mixed with them, which will swell.)
2. Tease a fresh specimen in glycerine for preservation.
3. **Transverse Section**; mount in glycerine, after being stained with picro-carmine. The fibres in section have a *polygonal* appearance, and are yellow in tint. (The small quantity of red tissue is that fibrous tissue mentioned above.)

II. White Fibrous Tissue.

Here again specimens will not consist only of white fibres, a number of connective tissue corpuscles being mixed with them: these two elements constitute the structure of *tendon.*

1. **Tendon,** from the tail of a mouse. Remove the skin from the extreme end of the tail and pull off a vertebra with the thumb-nail and forefinger; an attached fibre will be removed. Stretch this across the slide. The middle portion is to be covered with a drop of normal saline, the fibres gently separated at this part with needles, and the coverslip applied.

   The *white* fibres have a fine, delicate, *wavy* appearance, running in *parallel* rows. Sketch and compare with (I.).

   Add some 5 % acetic acid, they will gradually *swell*, becoming *indistinct*, while rows of *connective tissue corpuscles* appear having a square outline and distinct *nuclei*.
2. Tease in glycerine a piece of tendon which has been kept 24 hours in picric acid: examine under H.P. The fibres will be seen to have broken up into a number of fine *fibrillæ*.
3. **Longitudinal section of tendon** (tendo Achillis of dog): stain in logwood and mount in C. Balsam.

   The *corpuscles* will be seen arranged in parallel rows and *nucleated*. At parts deeper tinted, longitudinal markings appear on the corpuscles, from seeing through a thicker piece of the cell substance, as will be explained from the next section. The fibres do not stain well.
4. **Transverse section of tendon.** (Tendo Achillis of dog, or tail of young mouse; in latter case the bone, blood vessels, etc., must be disregarded.) Logwood; C. Balsam.

   L.P. The *fibres* appear transversely cut and bound together in *fasciculi* by small bundles of areolar tissue.

   H.P. The fasciculi contain deeply stained, irregular bodies throwing off numerous thin branches: these are the *corpuscles* in transverse section, their branches surrounding the *fibres* of the tissue.

III. Connective Tissue Corpuscles.

1. **Cornea of frog.** Mount flat in glycerine a gold preparation of the frog's cornea. Disregard the fine nerve fibres stretching and branching in various directions, but study carefully the well-stained *corpuscles*. The latter have large *granular* bodies and throw off *numerous branches*, those of neighbouring corpuscles appearing to be connected.

2. **Tadpole's tail.** Tease in glycerine a piece from the thinner portion. A better method is to cut off a piece of the tail, divide by scissors along the muscular median line, and split open one of these halves by needles: when split the two layers of epithelium should lie down on the glass slide and the connective tissue substance of the tail can be readily examined, after covering with a slip. This should be done in glycerine.

Between the developing blood vessels, numerous *connective tissue corpuscles* will be seen: they are well *branched*, granular and *nucleated*, many possessing a good amount of pigment.

IV. Areolar Tissue (Connective Tissue).

1. **Sub-cutaneous tissue.** Here all the factors will be well distinguished. From under the skin of a freshly-killed animal (rat) snip off a piece of the light fibrous tissue, by lifting it up with a pair of forceps and cutting a short distance below with a pair of scissors. Quickly place on a clean slide and spread out with needles, very gently breathing on it now and again to keep it moist. Mount directly by placing a drop of normal saline upon a cover-slip and covering.

Examine under H.P. with a small diaphragm. Numerous bands of *white fibres* will be seen running in various directions and possessing their characteristic wavy appearance. Not so distinctly, the yellow *elastic fibres* appear: they are less in number, very fine, and connected at times by branches. With care, *connective tissue corpuscles* may also be seen, showing their irregular branches.

Add a small quantity of 5 % acetic acid, and the white fibres swell, gradually disappearing, while the elastic fibres and corpuscles stand out more distinctly. At points bodies resembling white blood corpuscles may be seen, *leucocytes.*

V. Adipose or Fatty Tissue.

This modified form of connective tissue is best examined in specimens containing comparatively few fat cells, as parts of the mesentery, omentum, etc.

1. **Omentum.** Carefully spread out in normal saline, a piece of omentum from a freshly killed rat; cover, and

selecting a part which does not contain much fat, examine under L.P. and H.P. (N.B.—If a rat be used care must be taken not to mistake the pancreas, which spreads over the mesentery, for fat.)

With L.P. the *fat cells* will be distinguished as *large*, bright, *highly refracting bodies* mixed with the tissue fibres.

Under H.P. one can distinguish the fibres and see the individual cells with a *distinct* and oval outline. In some a small amount of granular protoplasm may be seen between the cell-wall and the outline of the fat globule or globules.

2. Mount in C. Balsam another piece of omentum which has been in alcohol for 24 hours, stained with logwood and passed through absolute alcohol, then turpentine and kreosote (page 6). The fat will have been dissolved from the cells, which under H.P. show the *cell-wall* and *nucleus*; the latter, during the formation of the fat, may have been pressed towards the wall, which also may have become irregular in outline during preparation. The cells will be seen to be embedded in connective tissue.

VI. Adenoid, Lymphoid or Retiform Tissue.

1. **Lymphatic Gland.** Gently shake in water and mount in glycerine a very thin section of fresh lymphatic gland, cut with the freezing microtome.

H.P. Disregarding the coat and trabeculæ of fibrous tissue, notice the fine *reticulum* of branching connective tissue *corpuscles*. Their *small* bodies throw off many filamentous *processes*, which joining those of neighbouring cells form a reticulum. The meshes are seen to contain leucocytes—and the shaking in water is adopted to remove as many as possible.

Demonstrations.

1. Silver preparation of tendon, to demonstrate the corpuscles by "negative images."
2. Reduction of *osmic acid* by fatty bodies and its use for demonstrating fat cells.
3. Development of fat cells.

V.—CARTILAGE.

Cartilage consists essentially of *cells* embedded in a *matrix* or ground substance, and its chief forms arise from variations in the structure of the latter. The *hyaline cartilages* have a transparent or slightly granular matrix, aud the different kinds may be distinguished by the arrangement of the cells. In *yellow elastic cartilage* the matrix is permeated by fine fibrillæ of elastic tissue; and *white fibro-cartilage* has a matrix showing white fibrous tissue.

I. Hyaline Cartilage.

1. **Shoulder-girdle of the newt,** or the transparent portion of the **frog's sternum,** are good examples of simple hyaline cartilage. Snip off a small piece from a freshly killed animal, scrape gently, to remove any adhering tissue, and mount in normal saline.

   Under H.P. a hyaline or very finely granular *matrix* will appear, studded with oval *cells* or corpuscles filling the *cell spaces* and consisting of granular *protoplasm* with one or two *nuclei.* In some parts, neighbouring cells will have their proximal sides flattened.

   Run in a little acetic acid; the cells will shrink away from the cell spaces, their protoplasm become granular and their nuclei appear very distinctly.

   Remove the specimen, wash it well in normal saline and place for a few minutes in logwood (or hæmatoxylin), wash again and mount in glycerine. The *nuclei* become very distinctly stained and the matrix slightly so.

2. **Articular cartilage.** Take a section, vertical to the articular surface and including a small piece of the (softened) bone: stain with eosin and mount in glycerine.

   L.P. Note the smooth free surface of the cartilage, and also its line of junction with the bone: the cells are distributed throughout the matrix, but get closer together towards the surface.

   H.P. The matrix is clear and hyaline; the cells in the deeper portion, near the bone, are *round, granular, nucleated* and frequently *grouped* from cell division, but on approaching the free surface they become *flattened*

in a direction parallel to the articular surface and the amount of matrix between them decreases. The cell substance is *granular* and the nuclei are distinct.

3. **Head of frog's femur.** Section vertical to the surface and treated as above (2).

In this specimen the arrangement and flattening of the cells, as the surface is reached, can be clearly seen, and the process of *cell division* is most distinct: the two or more *daughter cells* from division of the *mother cell.*

4. **Costal cartilage;** logwood, glycerine. H.P. The chief points to notice are, the very distinct *grouping* of the cells and the presence of fat-globules in their protoplasm. At the surface of the cartilage the cells become flattened.

5. **Parenchymatous cartilage.** Carefully remove the skin from both sides of the ear of a mouse; clean from adhering tissue and mount in glycerine.

L.P. The specimen *appears cellular* from the *small* amount of matrix: at places holes are seen in the cartilage.

H.P. Note the *very* small amount of matrix and the consequent approximation of the cells, giving rise to the name, *parenchymatous.* The cells are similar to those of former specimens.

II. Yellow Elastic Cartilage.

1. **Epiglottis** (or pig's ear). Stain a section with picro-carmine and mount in glycerine.

L.P. shows a *matrix* (yellow) containing many *cells* (red).

H.P. The matrix appears clear just round the cell-spaces but granular in other regions; if carefully studied this granular appearance is seen due to fine *fibrillations* of yellow elastic tissue.

2. **Arytenoid cartilage** (sheep); take a section through the point of attachment of the vocal cords, and parallel to their direction; stain with picro-carmine and mount in glycerine.

L.P. The cartilage at one portion of the section appears hyaline, at the other end fibrous.

H.P. Examine the section at the junction of the above parts and fine *elastic fibres* will be very distinctly seen, running from the denser elastic cartilage to the

hyaline matrix of the other portion. Trace these fibres and study their denser packing on approaching the cords. The cartilage *cells* and *nuclei* stain with the carmine.

III. White-Fibro Cartilage.

1. **Section of ligamentum teres and head of femur.** This is to be made parallel to the direction of the fibres of the ligament, stained with carmine and mounted in glycerine.

   L.P. *White fibrous tissue* gradually passing into the *cartilage* can be clearly seen: select that portion showing the transition and examine under H.P.

   H.P. At the above point the matrix is seen to consist of a number of *parallel fibres* similar to those of (II. 1) in connective tissue. The cells however are distinctly *cartilage cells*, round, clear and *nucleated*, but arranged in rows parallel to the direction of the fibres; they stain well in carmine. Follow the specimen towards the articular surface at the head of the bone, the cells become irregularly arranged and an articular cartilage is found; in the opposite direction the cells take the appearance of the connective tissue corpuscles found in tendon (II. 1 of connective tissue).

2. **Interarticular cartilage** (intervertebral). Cut a vertical section through two neighbouring vertebræ, stain with carmine and mount in glycerine.

   Examine under H.P.; the *cartilages* on the surface of the neighbouring vertebræ will appear and the *fibrous tissue* passing between them. Here the *fibro-cartilage*, close to the bone and with the appearances above described, may be seen.

VI.—BONE.

Specimens of bone when examined may be seen in a close, dense form known as *compact* bone; or of a less dense form, consisting of a number of slender bars or

spicula joining here and there to form a network, and then spoken of as *spongy* or *cancellated* bone. These pass gradually from one form to another, and in minute structure are seen to consist of the same elements.

To detect the histological structure, specimens of hard and of softened bone must be examined.

I. Hard Bone.

1. **Transverse section of long (compact) bone.** Place under L.P., and note the large *medullary canal* surrounded by a ring of *osseous tissue*. The latter will show a number of round, *dark* spaces distributed throughout its substance; they are the transverse sections of *Haversian canals*, tubules which in the living condition carry blood-vessels; they will vary in shape according to the relation of the plane of the section to the direction of the canals. A number of fine, concentric lines indicate the presence of the *lamellæ*, and with these are seen very small, dark, irregular spaces, the *lacunæ*. In this specimen the lamellæ are seen to be arranged in distinct systems :—

1. *Circumferential or Peripheral system*: placed at the outer surface of the bone, and in which the lamellæ run parallel to the outer surface.

2. *Haversian systems*: in which a number of concentric lamellæ are arranged round the various Haversian canals, the latter being placed in the centre.

3. *Perimedullary system*: where the lamellæ run parallel to the inner (medullary) surface of the bone. These are not always present, there being irregular *Haversian spaces* around which the lamellæ are placed.

4. *Intersystemic*: where rows of lamellæ occupy irregular spaces between the Haversian systems.

Examine an Haversian system under the H.P. Arranged with the lamellæ are the dark (air-filled) *lacunæ*, generally elongated in the direction of the former, very irregular in outline and giving off in various directions fine branches, *canaliculi*. On carefully tracing the canaliculi, those of neighbouring lacunæ will be seen to anastomose, while some from the row of lacunæ placed next to an Haversian canal open into that cavity. Careful sketches must now be made under both L.P. and H.P.

2. **Longitudinal section of long (compact) bone.** This is to be examined in a similar manner to the above. The chief points to notice are: the direction of the lamellæ, generally parallel to the long axis of the bone; and the Haversian canals. The latter run along the bone somewhat parallel to each other, and are joined by transverse branches.
3. **Section of frontal bone.** Examine as above. L.P. shows two peripheral plates of *compact*, enclosing a mass of *cancellated* bone.

   The H.P. will show the ultimate structure of the different parts to be similar to that of sections 2 and 3.

II. Softened Bone.

1. **Transverse section of long bone.** Stain with picro-carmine, and mount in glycerine.

   Examine under L.P. and note the various parts as seen in the similar section of hard bone; from the method of preparation the lacunæ, canaliculi, and Haversian canals will not appear as dark spaces but as red-coloured parts in a less deeply tinted ground (bone substance).

   H.P. Carefully study a lacuna, which is occupied by a stained *bone corpuscle* sending *branches* into the canaliculi, and containing a distinctly stained *nucleus*. The Haversian canals are occupied by some fine *connective tissue* surrounding the section of a *blood vessel* (in parts of the specimen this section may have fallen out during the course of preparation).
2. **Longitudinal section of long bone**; picro-carmine, glycerine.

   Under L.P. and H.P. compare this section with the above, and examine the various tissues as described.
3. **Frontal bone**; carmine, and mount in C. Balsam. The bone substance is similar to the above. The spaces of the spongy bone are filled with marrow, and contain fat cells.

III. Sharpey's (perforating) fibres.

1. Carefully remove (under dilute glycerine in a capsule) a few lamellæ from the piece of a softened frontal bone, by holding down the lower part of an edge with forceps,

and tearing off the upper part with another pair; mount in glycerine with the lower surface uppermost; or

2. Tease off the outer lamellæ from a coarse vertical section of the same. At various parts of the specimens fine, elongated *Sharpey's fibres* will be seen, gradually passing to a fine point. In the last method of preparation the elongated, conical spaces occupied by these fibres may appear.

IV. Periosteum.

1. Adult bone. Place specimens II. 1 and 2 under the H.P. The outer surface will appear more deeply stained, the *periosteum*; this consists of connective tissue which next to the bone substance contains a greater proportion of yellow, elastic fibres.
2. Young bone: carmine, glycerine. Under H.P. the *periosteum* has a similar appearance to that above, but between the yellow elastic fibres and the bone substance a number of well stained granular corpuscles are seen, the *osteoblasts*.

V. Marrow.

Described as of two kinds, viz. yellow marrow and red marrow.

1. **Yellow marrow.** Remove a small piece from the inside of a fresh long bone and mount in a little normal saline. Under L.P. and H.P. it is seen to consist chiefly of *connective tissue* and *fat cells*.
2. **Red marrow.** Break across the long bone of a guinea-pig, and mount a small piece of the marrow in normal saline, surrounding with oil. Fat cells are few. Some *red-coloured* cells may be seen and also large indefinite-shaped *giant cells* (myeloplaxes) with large or several nuclei; while round *marrow cells* with clear protoplasm and nuclei appear.

VII.—MUSCLE.

I. Unstriated (Involuntary) Muscle.

1. Carefully and finely tease in glycerine a small piece of muscular tissue from the intestinal walls. This should have been placed in water for a short time after removal from the spirit (page 59).

   Under H.P. and with a small diaphragm. Notice the long, *spindle-shaped cells* of *unstriated muscle fibres*, each with a distinct, elongated *nucleus* placed at the centre and finely granular in appearance. Examine also groups of these cells and their arrangement.

2. **Frog's bladder.** Stain with logwood or carmine, and mount in C. Balsam, the specimen being carefully spread out to prevent creases.

   Examine with L.P. and H.P. Disregard the round nuclei of the connective tissue corpuscles and of the endothelial cells of the blood vessels. Note the elongated, *unstriated muscle fibres*, collected in small *bundles*, running in various directions, and containing the well stained *nuclei*.

3. **Transverse section** of the muscular coats of the small intestine, to show the fibres in section; stain with logwood, mount in C. Balsam.

   With H.P. note the two layers, one with the fibres cut longitudinally, the other transversely. The former have an appearance already described; the latter appear *round* or slightly angular, some containing a deeply stained spot where the razor has passed through the *nucleus*. This will not occur in every fibre, and the section should be compared with the transverse section of a medullated nerve fibre (page 26) to note the distinguishing points. Between the fibres a certain amount of fine tissue may be seen.

II. Striated (Voluntary) Muscle.

1. **Fresh muscle.** Remove a small piece from the thigh of a freshly killed frog and tease carefully, but well, in normal saline; cover and examine under L.P. There are numerous *muscle-fibres* embedded in fine connective tissue (endomysium).

Under H.P. carefully study an individual *fibre*; it has a *striated* appearance from the presence of alternate light and dark bands in a transverse direction. Here and there the muscle substance will have shrunk away from its sheath or *sarcolemma*, which shows as a fine distinct line parallel to the direction of the fibre; or in places the muscle substance will have been broken across and this sarcolemma will appear as two distinct lines joining the outer edges of the separated pieces.

Sketch the above, and then run in a little acetic acid; in a short time the mass becomes granular and *nuclei* appear distributed throughout its substance.

2. **Striations.** Tease in glycerine a piece of crab's muscle or a piece of water-beetle's muscle, kept a few days in spirit.

Under H.P. the *striations* from the alternate light and dark lines appear very clearly.

3. **Muscle fibrillæ.** Tease in glycerine a piece of muscle hardened in ·5 % chromic acid; or a piece of boiled meat. The fibres will be seen to have split longitudinally, into a number of very fine *fibrillæ*, which still show the *striations*.

4. **Muscle discs.** Tease and examine in a similar manner a small piece treated with 2 % hydrochloric acid. Here the fibres will be seen to have broken up *transversely*, forming the muscle *discs*.

5. **Transverse section.** Stain with logwood and mount in C. Balsam a section of dog's muscle.

L.P. shows the *fibres* in section, with connective tissue between them (endomysium). The fibres are collected in bundles forming *fasciculi*.

H.P. The fibres appear as definite elements, showing a clear outline, the *sarcolemma*, inside and close to which lie the well stained *nuclei*. Within the areolar tissue between the fibres, blood vessels may be seen in section.

III. Cardiac Muscle.

1. Tease in glycerine a piece of cardiac muscle, previously stained with picro-carmine. Under the L.P. pick out a few well separated fibres and examine under H.P.

Here and there the cells may be seen to give off a small *branch*; they are but slightly greater in length

than in breadth; their *striations* are much less distinct and regular than in ordinary striated muscle; they possess *no sarcolemma* and the *nuclei* are oval, centrally placed with but *one* (or at most two) to each cell. The cells are joined end to end and this junction may be clearly distinguished.

VIII.—NERVOUS TISSUES.

I. Medullated Nerve Fibres.

1. **Fresh nerve.** Remove a small piece from the sciatic (or other large nerve) of a freshly-killed frog; spread out and quickly tease upon a dry slide, by holding one end with a needle and working from this end in the direction of the fibres with another needle; add a drop of normal saline, cover, and examine with L.P.

   The nerve is seen to be formed of *fibres* bound together by fine *areolar tissue.*

   H.P. Each *fibre* is distinctly marked, and possesses a highly refracting *medullary sheath* (white substance of Schwann) which escapes at the end of a cut fibre in fatty globules. This sheath is covered on the outside by a fine membrane, the *primitive sheath* (sheath of Schwann) which with care may be distinguished at different points as a fine line.

2. Spread out a fresh piece of nerve in the same manner, but add a drop or two of chloroform (instead of normal saline) and cover, being careful during the examination that all the chloroform does not evaporate. This dissolves the fatty substance of the medullary sheath.

   H.P. The *primitive sheath* may be distinctly seen, and running along the middle of the fibre appears a granular line, the *axis-cylinder.*

3. Tease in glycerine some frog's nerve, slightly teased and allowed to remain in 1 % osmic acid for about two hours. Select under L.P. a long and distinct fibre.

H.P. Study the *medullary sheath*, which is stained by the osmic acid and appears broken at short intervals; within this lies the lighter *axis cylinder*. Trace along the fibre and at certain points a constriction appears, the medullary sheath stops while the axis cylinder passes, a *node of Ranvier*. About midway between two nodes of Ranvier, and lying just beneath the primitive sheath, the unstained *nucleus* (surrounded by a little granular protoplasm) may be seen projecting into the dark medullary sheath.

4. **Transverse section of a nerve** (large). Stain with logwood, mount in C. Balsam. During the mounting the fatty substance of the medullary sheaths will have been dissolved.

L.P. The section is seen to be built up of separate nerves, each bounded by a fine line, a section of the *perineurium*, and held together by connective tissue, *epineurium;* the nerves consisting of *nerve fibres* with *endoneurium* between them.

H.P. The section of each *nerve fibre* shows a fine circular line, *primitive sheath*, enclosing a space occupied by the *medullary sheath*, in which lies a deeply-stained spot, the divided *axis-cylinder*. Compare with the transverse section of unstriated muscle fibres (page 23).

II. Non-Medullated Nerve Fibres.

1. Remove the nerve from the fresh **spleen** of an ox, carefully tease in glycerine, and examine under H.P. Note few medullated and many *non-medullated nerve fibres;* the latter are pale and granular, each showing numerous oval *nuclei*, a *primitive sheath* and an *axis cylinder*.
2. **Cornea.** Examine under H.P. the gold preparation of the frog's cornea (page 14). The nerve fibres will be seen, *non-medullated* and dividing into many branches, the finest of which show numerous slight thickenings along their length—*varicose nerve fibres*. The finest branches have no primitive sheath.

III. Nerve Cells.

1. **Spinal cord.** Tease well in glycerine a piece from the anterior cornu of the lumbar region (ox) previously stained with carmine.

L.P. Note the fine mass of connective tissue, nerve fibres, and large, branching, *multipolar nerve cells.*

H.P. Examine a nerve cell: it consists of a granular *cell body* throwing off numerous dividing *branches*, and a large distinct *nucleus* in which lies a deeper stained *nucleolus.* Sketch under both L.P. and H.P.

2. Mount in C. Balsam, a section of the ganglion on the posterior root of a spinal nerve, stained in carmine.

L.P. Disregard the nerve fibres and areolar tissue, but study the collection of ganglionic cells.

H.P. Each cell possesses a *nucleus* and *nucleolus*, and is enclosed in a *nucleated capsule;* some may be seen to give off a branch.

Demonstrations.

1. Silver preparation to show the *nodes of Ranvier.*

2. Transverse section of medullated nerve fibre treated with osmic acid, to show the cyliudrical nature of the medullary sheath.

3. *Unipolar* nerve cells of Bidder's ganglion in the frog's heart.

IX.—BLOOD VESSELS.

I. Arteries.

1. Medium sized artery. Transverse section; logwood, C. Balsam.

L.P. Note the three coats:—

1. *Inner coat*, appears clear and narrow.

2. *Middle coat*, broader and well stained.

3. *Outer coat*, not well stained and irregular in limitation.

H.P. The inner coat may show at its inner edge a few *nuclei* of the *endothelial cells* which line it; beneath these lies a clear line of regular thickness, the layer of *yellow elastic fibres.*

Of the middle coat, the *circularly* arranged, *unstriated muscular fibres* with their distinct *nuclei* may be seen, and between these a certain amount of *connective tissue* appears in wavy lines.

The outer coat is less stained, and it is built of fibres of *connective tissue*, between which the connective tissue corpuscles appear by their nuclei, which stain well.

2. **Large artery.** Transverse section, stained with picro-carmine, mount in C. Balsam.

L.P. Compared with the medium sized artery it is seen to have the same number of coats; the middle one is, however, comparatively less thick, the outer one thicker.

H.P. Distinguish the various elements as in 1, and note the greater amount of connective tissue compared with the muscle fibres in the middle coat.

3. **Arterioles.** Stain with carmine a specimen of **pia mater** removed from a young animal and hardened; mount in C. Balsam.

H.P. Disregard the areolar tissue and study the small arteries; note the elongated *nuclei* of their *muscular fibres* arranged transversely; in the smallest only one layer is seen. By careful focusing the more *circular nuclei* of the endothelial cells lining the arteries, may be distinguished.

4. **Endothelial cells** lining the arteries. Carefully spread and mount in C. Balsam a specimen of frog's bladder in which the blood-vessels have been injected with silver nitrate (page 61).

H.P. Note the smallest vessels (capillaries) and larger ones (arteries and veins). Examine the *endothelial cells* of the arteries, indicated by the black outlines; they are more elongated compared with those of the veins. The transverse lines seen crossing the vessels are indications of the muscular fibres (3).

5. **Fenestrated membrane** or layer of elastic fibres, underlying the sub-endothelial layer. Carefully tear off the inner coat of an artery from a large animal, and after cleaning, tease in glycerine.

H.P. It consists of a layer of yellow *elastic fibres*, which at times form a membrane with numerous holes (fenestrated).

II. Capillaries.

1. **Frog's bladder.** Examine under H.P. the silver preparation I. 4. Choose a small artery, and gradually

trace its finer and finer branches till they open into capillaries. These are distinguished by not showing transverse lines to indicate muscle fibres, having but a single layer of *endothelial cells* as constituting their whole structure. At points the intercellular substance (which is the dark part reducing the silver nitrate) appears as a dark patch. Trace the capillaries in the same direction and they will be seen to collect and open into veins.

2. **Pia mater.** Examine under H.P. specimen I. 3, and trace, as before, an artery till it opens into *capillaries*, indicated by the absence of the transverse nuclei of muscle fibres; these fine tubes show very distinctly the stained *nuclei* of their endothelial cells.

III. Veins.

1. **Transverse section of medium sized vein**; stain in logwood, mount in C. Balsam.

   L.P. The thickness of the walls is much less than that of an artery of the same calibre; but the *internal*, *middle*, and *outer coats* still appear.

   H.P. In the internal coat the *nuclei* of the *endothelial* cells and the layer of *elastic tissue* may be seen. The middle coat shows *muscular fibres* with their stained *nuclei*, but they are mixed with a great deal of *connective tissue*; and the line of demarcation between the middle coat and the outer one is far less distinct than in the artery.

2. **Endothelial cells**: examine the silver preparation of frog's bladder, I. 4, under H.P. The *endothelial cells* are very distinct, but are broader than those of the arteries.

X.—ALIMENTARY CANAL.

I.—Tongue.

1. **Tongue of cat**: vertical section through the upper portion; logwood, C. Balsam.

   L.P. An *epithelial* covering surrounding a mass of *striated muscle* fibres embedded in connective tissue. At points the surface of the epithelium forms a number of *papillæ*.

H.P. Epithelium is seen to be *stratified*, the lower cells being somewhat columnar and gradually passing to flat ones at the surface; *nuclei* show well. Separated from the epithelium by areolar tissue, and surrounded by the same, are bundles of *striated muscles* which are cut in various directions and show distinct *nuclei*. At points *mucous glands* may be seen.

II.—Tooth.

1. **Vertical section** of human tooth. Under L.P. it is seen to consist of a *pulp cavity* surrounded by *dentine*; the latter is marked with wavy, radial lines, and is covered at its upper portion by a sheet of *enamel*, and by the *crusta petrosa* at the lower part: each narrowing gradually to their line of junction.

   H.P. The Enamel consists of (polyhedral) *prisms* arranged perpendicularly to the surface of the dentine: concentric lines may be seen passing across them in a direction somewhat parallel to the surface.

   The radial lines of the Dentine are seen to be fine *tubules* opening from the pulp cavity and passing through the *matrix* of the dentine towards the crusta petrosa or the enamel; sometimes dividing *dichotomously*, gradually growing finer during their passage, which is in wavy lines, the latter being small secondary waves placed upon larger primary ones. At times transverse *incremental lines* appear, which are formed of *interglobular spaces* having outlines of imperfect circular curves.

   The Crusta Petrosa will show *lacunæ* and *canaliculi* as in bone, but the former are much more irregular and no Haversian canals are seen.
2. **Transverse sections** of various parts are to be compared with the above.

III. Salivary Glands.

1. **Mucous gland**: submaxillary gland of dog; logwood, C. Balsam.

   L.P. Gland is seen to consist of a number of *ducts* and *alveoli* embedded in fine *connective tissue*, which contains many blood-vessels.

   Select a thin portion and examine under H.P. Ducts: consist of a single layer of *columnar* epithelium cells,

each having a centrally placed *nucleus* and protoplasm *granular* towards the aperture of the duct but faintly *striated* with radial lines at the peripheral portion.

The alveoli are built of *two* kinds of cells: chiefly of cells somewhat pear-shaped, *not well stained* and with a distinct nucleus in the *corner* (characteristic of *mucous cells*). One or more of the second kind may be seen at the periphery of an alveolus; having *well stained, granular* protoplasm and a *centrally placed* nucleus—the *demi-lunes.*

2. **Serous gland:** parotid gland of dog; logwood, C. Balsam.

Examine under L.P. for ducts, alveoli, etc., as above.

H.P. Ducts have same structure as before. The alveoli are formed by groups of *one* kind of cell; each having *well stained, granular* protoplasm with a *centrally* placed nucleus (characteristic of *serous cells*).

Compare well the two classes of mucous and serous cells.

IV. Tonsils.

1. **Section of human tonsil;** logwood, C. Balsam.

L.P. The *epithelial* coat may be seen, under which lies a layer of *connective tissue* throwing off many branches, while beneath the connective tissue are *mucous glands* and numerous circular patches, the *lymph follicles.*

H.P. Examine the character of each of these structures; particularly the follicles, built of *adenoid tissue* enclosing *leucocytes* with well stained nuclei.

V. Œsophagus.

1. **Rabbit,** transverse section: logwood, C. Balsam.

L.P. Note the three distinct coats (1) *epithelial,* (2) *sub-epithelial,* (3) *muscular.*

H.P. Epithelial coat formed of *stratified* epithelium, cells *nucleated* and passing from lower columnar to superficial flattened ones; lower border has a wavy outline.

Sub-epithelial coat formed of *connective tissue,* containing numerous blood-vessels.

The muscular coat is formed of layers of *striated muscle* (being from the upper portion of the œsophagus) embedded in connective tissue.

2. **Dog.** Transverse section; carmine, C. Balsam. Similar to that of the rabbit, but the sub-epithelial coat contains a number of *mucous glands* opening by *ducts* upon the surface.

VI. Stomach.

1. **Cardiac end**: dog; carmine, C. Balsam.

L.P. Four distinct coats (1) *mucous membrane*, (2) *sub-mucous coat*, (3) *muscular coat* of several layers, (4) *external coat.*

Examine carefully each coat under H.P.

(1) Mucous coat. Formed of simple *tubular glands* with fine adenoid tissue between them. They possess *three* kinds of cells: *columnar* cells forming the mouth and neck, with well stained nuclei placed towards the base; *peptic* (*chief* or *central*) cells, forming the principal part of the remainder of the gland, are well coloured granular cells, each with a central nucleus; *ovoid* (*parietal*) cells, less in number, are mixed with the peptic cells, but chiefly at the junction of the latter with the columnar; they are oval and possess large oval nuclei. The *muscularis mucosæ*, formed of a thin layer of *unstriated* muscular tissue, is placed at the base of the glands.

(2) Sub-mucous coat: formed of fine *areolar tissue*, containing numerous *blood vessels.*

(3) Muscular coat: possesses *three* distinct layers of *unstriated* muscular fibres, the transverse, oblique, and longitudinal.

(4) External coat shows as a clear outline (section of endothelial cells) between which and the muscular coat lies some fine connective tissue.

2. A similar section, stained with aniline blue, is to be mounted in glycerine, and examined under H.P., when the *ovoid cells*, which stain well, may be distinguished from the chief or peptic cells.

3. **Pyloric end**; dog, logwood, C. Balsam.

L.P. shows the same coats as with the cardiac end, but the glands are more branched, and have longer ducts with wider mouths.

H.P. The glands are built of *two* kinds of cells, viz., the *columnar* cells, lining the ducts and similar in character to those at the cardiac end; and cells which do *not stain* well, are more cubical, and have their nuclei at the *peripheral portion*. This part of the gland is often seen cut transversely, and has a good amount of fine connective tissue surrounding it.

VII. Small Intestine.

1. **Transverse section**; dog, logwood, C. Balsam.

   L.P. Note the *four coats*, similar to those of the stomach except that the muscular one is formed of only *two* layers, viz., the *internal circular* and *external longitudinal* (which in this specimen will show their fibres cut respectively longitudinally and transversely). The mucous coat contains simple glands, *glands of Lieberkühn*, and projections, the *villi*. In the upper part of the small intestine may be seen, in addition to the above, *Brünner's glands*, compound glands whose acini lie in the submucous coat, and whose ducts pierce the muscularis mucosæ to open on the surface.

   H.P. Each *villus* is formed of a core of adenoid tissue containing fine *blood vessels* and a *lacteal* in the centre, indicated by fine lines. It is covered by a single layer of *columnar* epithelium cells, which are granular, have a distinct *nucleus* and fine *clear outer border*; many undergo mucous degeneration and form swollen, unstained *goblet cells*, the nuclei being pressed to the edge. *The glands of Lieberkühn* are simple crypts, formed of cells showing a distinct nucleus. Beneath these glands is the *muscularis mucosæ*.

   The characters of the other layers are similar to those described for the stomach.

2. Tease in normal saline a small piece of mucous membrane from the frog's intestine, and study the characters of the *columnar*, *goblet* and *gland cells* described above.

3. Section of mucous membrane, *parallel to the surface*, from dog; logwood, C. Balsam.

   H.P. shows transverse sections of *glands of Lieberkühn*, with the *lumen* surrounded by cells and the interglandular adenoid tissue. In many places the glands have slipped

out, showing only the adenoid tissue and a space. Transverse sections of *villi* may at times occur. (Section to be compared with a tangential one from the medulla of the kidney.)

4. **Peyer's patch**; logwood, C. Balsam.

L.P. shows the *villi* and *glands* of the small intestine, but in the tissue of the mucous layer lie several masses of *adenoid tissue*, round in shape, causing a bulging of the mucous membrane, and not covered by villi or glands. When they occur alone they are called *solitary glands*.

VIII. Large Intestine.

1. **Transverse section**; dog; logwood, C. Balsam.

L.P. shows coats similar to those of the small intestine, but with *no villi* and no Brunner's glands.

H.P. The glands are the same in structure as the *glands of Lieberkühn*; they contain generally a greater number of goblet cells.

XI.—LIVER.

I. Fresh Liver.

1. Gently scrape the cut surface of a fresh rat's liver and mount in normal saline; examine under L.P. and H.P.

Disregarding the number of blood corpuscles, carefully study the *hepatic cells*; note their very *granular protoplasm* with no cell wall, their *polygonal shape* and centrally placed *nucleus*: sketch.

Run in a little 1 % acetic acid to bring out the nuclei distinctly.

2. Scrape and mount in a similar manner the hepatic cells from a frog's liver. Note with H.P. the large and small *fat globules* they contain.

II. Hardened Liver.

1. **Pig's liver**; logwood, C. Balsam.

L.P. Note the polygonal hepatic *lobules* (very distinctly marked in the pig) surrounded by connective tissue forming the *capsule of Glisson*, which is continuous

with the outer coat of the liver. Within this tissue and between the lobules lie: the *interlobular veins* (branches of the portal vein) with large lumen but thin walls: the fine branches of the *hepatic arteries* having small lumen but stout walls: and the *bile ducts* lined with columnar epithelium. In the centre of the lobules lie the *intralobular veins*, and at parts the large *sublobular veins* may be seen in section.

H.P. The polyhedral *hepatic cells* with their *nuclei* are stained by the logwood; note their *radial* arrangement from the intralobular veins towards the circumference of the lobule, with the (crushed) capillaries between them (*cf.* injected specimen). The coats of the arteries, veins and ducts may be distinctly seen and studied.

2. **Dog's liver**; logwood and C. Balsam.

Examine under L.P. and H.P. for parts described above; note the small amount of interlobular connective tissue, which is here found chiefly round the branches of the portal vein (interlobular), hepatic artery and duct: these three run together as in the pig. The *intralobular* join to form the *sublobular*, which join to form the *hepatic veins*.

III. Injected Liver.

Rabbit, injected with carmine; mount in C. Balsam.

Study under L.P. the interlobular and intralobular veins and then under H.P. their connection by the *capillaries*, between which lie the hepatic cells.

XII.—LUNG.

I. Trachea.

1. **Transverse section**; picro-carmine, C. Balsam.

L.P. Note the following layers—(1) *Epithelial* layer, (2) *Connective tissue*, (3) Incomplete *ring of cartilage* joined at the ends by *unstriated muscle* fibres, (4) *Connective tissue*.

Carefully examine the various layers under H.P.

1. Epithelium; *stratified*, the outer cells being *columnar* and *ciliated*; beneath these lie some fine *adenoid tissue* and a layer of *yellow elastic fibres*, the latter run longitudinally and therefore appear in transverse section (*cf.* connective tissue, page 13).

2. Connective tissue; this holds *mucous glands* which open upon the surface by *ducts*. The tissue becomes denser on approaching the cartilage rings.

3. Cartilage rings; are formed of *hyaline* cartilage, the cells being closely grouped. The ends of the rings are joined by *unstriated muscle* fibres, arranged transversely.

4. Connective tissue; very fibrous close to the cartilage rings, and holds numerous blood vessels.

2. **Longitudinal section**; logwood, C. Balsam. The section is carried some distance so as to include several cartilage rings, and is to be carefully compared with the transverse section, particularly with regard to the layer of elastic fibres under the epithelium. Examine the parts mentioned in the former section, under L.P. and H.P.

II. Lung.

1. **Stained mammalian lung**; section, carmine, C. Balsam.

L.P. Note the sections of *arteries, veins, bronchial tubes*, and general *lung tissue.*

H.P. The bronchial tubes possess the same coats as the trachea, but differ, firstly in the cartilage being arranged in *irregular pieces* round the tube, and secondly in the unstriated muscle forming one *continuous ring* round the tube, lying just beneath the layer of yellow elastic fibres.

Next to the sections of large bronchial tubes, those of the arteries and veins appear.

The general lung tissue shows sections of the *alveoli* lined by a single layer of *epithelial cells*, whose *nuclei* stain well and appear either in section, or in the cell when the side of an alveolus can be seen superficially. Between neighbouring alveoli (crushed) blood *capillaries* appear, also some very fine *connective tissue.*

2. **Unstained mammalian lung**, glycerine. Study the same parts of this section under L.P. and H.P.
3. **Injected lung** (carmine injection) mount in C. Balsam.
   Examine under L.P. and H.P. to show the arrangement of *blood vessels* and *capillaries* on the walls of the alveoli; the capillaries are large, freely anastomose, and leave but small spaces between them.
4. **Newt's lung**; gold preparation, mount in glycerine. This is to demonstrate the *epithelium* lining the lungs.

XIII.—LYMPHATIC SYSTEM.

I. **Lymphatic Vessels** (demonstrations).

1. **Lymphatics of diaphragm** (rabbit; pleural side), silver preparation.
   With L.P. note the numerous inter-communicating light spaces (compared with the tint of the other tissues) —the *lymphatic vessels.*
   H.P. The vessels are seen to be lined by a single layer of *endothelial cells*, similar to those seen in the silver preparation of blood vessels in the frog's bladder. In the larger vessels transverse lines, indicating the *valves*, may be seen.
2. **Septum cysternæ lymphaticæ magnæ** of the frog; prepared with silver nitrate.
   Under H.P. by alteration of the focus, *two* layers of *endothelial cells* may be seen, one covering the membrane on the side towards the posterior lymph sac, the other on the side towards the peritoneal cavity. At certain points distinct openings or *stomata* appear, allowing communication between the above cavities, they are surrounded by a layer of *granular cells.* The wavy outlines of the endothelial cells are to be noticed, and also the small, dark, irregular patches sometimes found at the junction of the cells, the *pseudostomata.*

II. Lymphatic Glands.

1. **Simple.** Examine the sections of the tonsils (p. 31) or Peyer's patch, p. 34). Note the circular mass of *adenoid tissue* (lymphoid tissue) denser towards the periphery, and enclosing in the meshes leucocytes, whose nuclei stain well.

2. **Compound**; dog; logwood, C. Balsam.

L.P. The gland is surrounded by a *capsule* of fibrous tissue, sending in *trabeculæ*, which in the outer portion or *cortex* of the gland are radial, but in the inner portion or *medulla* form an irregular meshwork. The substance of the gland is composed of *adenoid tissue*, which towards each trabecula is less dense in form, contains but few leucocytes and stains lightly, the *lymph sinus;* but further away it is denser, well filled with leucocytes and stains deeply.

H.P. The capsule and trabeculæ are seen to be composed of *fibrous tissue*, and contain some fibres of *unstriated muscle.* In the lymph sinus carefully note the *branching, nucleated* cells forming the adenoid tissue with but few leucocytes in the meshes; also the numerous leucocytes in the denser portions.

Demonstration.

Silver and logwood preparation of lymphatic gland, to show the endothelial cells covering the trabeculæ.

III. Thymus Gland.

1. From child; logwood, C. Balsam.

L.P. shows a *capsule* of fibrous tissue sending in *septa*, which divide the body into *lobules* and again into *follicles.*

H.P. The follicles are separated by these fine septa, and consist of *adenoid* (or retiform) tissue containing *leucocytes.* The adenoid tissue is dense towards the outer portion or *cortex* of each follicle, but less dense at the *medulla*; here are found small bodies, which show a concentric striation and nuclei, they are called the *concentric corpuscles of Hassall.*

XIV.—SPLEEN.

Spleen of cat; logwood, C. Balsam.

L.P. shows a fibrous *capsule*, which throws in numerous *trabeculæ* dividing the spleen into irregular spaces; the latter are filled with the *splenic pulp* which has a blue and red appearance, the former due to the staining, the latter to the presence of blood. Round masses of a pure blue tint, *Malpighian bodies*, are distributed about the spleen substance.

H.P. The capsule (covered on the outside by a layer of endothelial cells, whose nuclei may be seen) and the trabeculæ are formed of *fibrous* tissue, with some *unstriated muscle* fibres and *blood vessels* seen in section.

The splenic pulp is of *adenoid tissue*, whose meshes are filled with *red* corpuscles and *leucocytes* (latter stained by the logwood.)

The Malpighian bodies are also built of *adenoid tissue*, denser towards their periphery, but the meshes simply contain *leucocytes*—hence their white colour in an unstained section and pure blue in the logwood stained one; in these bodies sections of blood vessels may be seen.

XV.—THE DUCTLESS GLANDS.

I Suprarenal Capsule.

1. Man; logwood, C. Balsam.

L.P. shows a *capsule* and a body divided into a *cortex* and *medulla*.

H.P. The capsule is of *fibrous tissue* throwing in a number of fine branches.

The cortex consists of three parts; the *outer zone*, of well nucleated cells, collected in small masses; a *middle zone* of columns of cells in cylindrical masses; the *inner zone* of irregularly grouped cells.

The medulla consists of masses of cells embedded in connective tissue, with numerous blood vessels.

2. Guinea-pig.
3. Rabbit. } Compare with above.

II. Thyroid Gland.

1. **Human**; logwood, C. Balsam.

L.P. shows a *capsule* which throws in branches to divide the gland into *lobes* and *lobules*.

H.P. The lobules contain *closed vesicles*, varying in size and shape, but all lined by a *single* layer of *nucleated flat* cells which rest on a basement membrane. Fine *connective tissue* is found between the vesicles; these may contain a stained substance, due to the hardening of the contents during preparation.

XVI.—KIDNEY.

I. Radial Section.

Radial Section of the Kidney of a small animal (guinea-pig), logwood, C. Balsam.

L.P. shows the kidney divided into two parts, viz.:—

1. *Cortical portion*, having an irregular appearance, from the convoluted tubes and the glomeruli; at intervals bundles of straighter tubules radiating towards the surface may be seen, the medullary rays.

2. *Medullary portion*, having a radial striation from the arrangement of the collecting tubules, which join dichotomously; it is divided into pyramids of Malpighi, upon the surfaces of which the tubules open into the pelvis (or calices and so to the pelvis) from which originates the ureter. (The tubules forming the medullary portion may have been cut transversely, and the radial arrangement not be so distinct).

II. Cortex.

1. **Radial section**; dog, carmine, C. Balsam.

L.P. Note the outer *capsule* of the kidney; the general irregular arrangement of the *tubules*, which,

however, at intervals shows tracts of more parallel arrangement running radially, the *medullary rays*; and the well stained *Malpighian corpuscles*.

H.P. The various parts are connected by fine *areolar tissue*, continuous with the fibrous capsule. Each *Malpighian corpuscle* consists of an outer *capsule* of nucleated, flat epithelial cells, and contains a bunch of capillaries forming the *glomerulus*; it opens by a narrow *neck* into the *proximal convoluted tubule*, which with the *distal convoluted tubule* form the chief structures of the cortex. The epithelium of each convoluted tubule is *nucleated, granular* at the inner portion and somewhat striated at the outer one. The distal convoluted tubules are connected by junctional tubules with the collecting ones; these show clear nucleated cells.

2. Divide a fresh kidney, gently scrape the cut surface of the cortex, mount these scrapings in normal saline and study under the H.P. for the various cells described above.

III. Medulla.

1. **Radial section**; logwood, C. Balsam.

L.P. The chief part is formed of the *collecting tubules*, which join dichotomously and open upon the surfaces of the pyramids of Malpighi.

H.P. The collecting tubules, which show a clear lumen, are formed of *clear, nucleated, cubical cells*, becoming *columnar* as they approach the apex of the pyramid. In that portion of the section towards the cortex are the *looped tubules of Henle* (which join the proximal and distal convoluted tubules) each formed of a *narrow descending limb* and a *broader ascending limb*; the epithelium of the former is clear, very flat with prominent nuclei; that of the latter is granular, nucleated, and at times imbricate.

2. **Tangential section**; logwood, C. Balsam.

Under H.P. study the various tubules and the character of their cells. In many cases the cells will have dropped out and the connective tissue is well seen. (Compare with the transverse section of the glands of Lieberkühn p. 33).

3. Fresh kidney; examine in normal saline under H.P. some scrapings from the medullary portions.

IV. Injected Kidney. (Blood vessels with carmine.)

Radial section, mount in C. Balsam.

L.P. Note the *interlobular arteries* running radially from the junction of the cortex and medulla towards the capsule, and giving off an *afferent vessel* to each Malpighian corpuscle, where it breaks up into the *glomerulus*. From this emerges an *efferent vessel* which breaks up again into a second system of *capillaries*, joining with those of other efferent vessels round the convoluted tubules; these finally collect to form an *interlobular vein* which runs parallel to an interlobular artery. The medulla is supplied by *arteriæ rectæ* which originate from the same branches of the renal artery that give rise to the interlobular arteries.

V. Bladder and Ureter.

1. **Bladder**; dog, logwood, C. Balsam.

L.P. shows three coats: the mucous, sub-mucous, and muscular coats.

H.P. The mucous coat is formed of *stratified epithelium*, the upper somewhat cubical, lower columnar or pear shaped cells; but the cells, fitting into one another, have an irregular appearance in section. The sub-mucous coat is formed of *connective tissue* and contains many *blood vessels*, seen in section. The muscular coat is formed of *unstriated muscle fibres*, placed in several layers separated by connective tissue.

2. **Ureter**; transverse section; logwood, C. Balsam.

L.P. shows similar coats to the bladder, but the muscular coat is distinctly divided into *three* layers, *two* with the fibres longitudinally arranged, and *one*, middle one, with circular fibres.

Examine the parts under H.P.

XVII.—SKIN AND HAIR.

I. Skin.

1. **Vertical section** from the tip of a finger; logwood, C. Balsam.

L.P. Note the division into two distinct layers; the *epidermis* which is again divided into an upper faintly stained part, and a lower deeply stained part and the *dermis*.

Study these various layers under the H.P.

Epidermis consists of several layers, viz.:

1. *Stratum corneum* (or horny layer). The cells are not distinguished as separate, are but very faintly stained, and with no nuclei visible.

2. *Stratum lucidum*, a clear narrow layer, formed of few cells, with stained flattened nuclei.

3. *Stratum Malpighii* (or rete mucosum) stains well, consisting of several layers of cells distinctly nucleated; the lower ones are somewhat columnar and arranged in a wavy line, being on the surfaces of the papillæ.

Dermis (cutis vera or corium). This gradually passes into the sub-cutaneous tissue. It is formed of dense *areolar tissue*, which is continued up into small prominences under the epidermis, called *papillæ*; some of these contain small oval bodies with well stained transverse *nuclei*, the *Tactile corpuscles*.

In the lower portions of the connective tissue *fat cells* and *blood vessels* are freely distributed.

Through the epidermis may be seen spiral *ducts*, leading to coiled tubes formed of cubic nucleated cells placed in the sub-cutaneous tissue, the *sudoriferous* or *sweat glands*. Again in some the lowest portions show sections of *Pacinian bodies*, appearing as oval masses of concentric lines.

2. **Skin of negro**, stain with eosin, C. Balsam.

Notice the same parts as in the above section, but also the presence of *pigment granules* in the lower cells of the epidermis.

3. **Nail**; transverse, vertical section; logwood, C. Balsam.

Note the thickening of the *stratum corneum* to form the nail, and the *long* papillæ of the dermis.

II. Hair

1. Remove a hair from the head and mount in C. Balsam.

   L.P. shows the *stem* generally surrounded at its base by the root sheath removed from the hair follicle.

   H.P. The hair consists of (1) *Cuticle*, a layer of imbricate, epidermic cells, giving a saw-like appearance to the edge. (2) *Cortex*, appearing homogeneous with a few longitudinal striations and containing fine pigment granules. (3) *Pith* or marrow, with fine air-spaces.

2. Rabbit, mount in C. Balsam.

   Note the regular arrangement of the cavities in the pith and the small amount of cortex.

3. Compare specimens from Sheep and Rat with the above.

4. **Verticle section of hair follicle**; logwood, C. Balsam.

   L.P. shows the hair *stem* arising from the *hair-bulb* surrounding a *papilla*, and passing out of the *hair follicle* by the *mouth*. This follicle dips down below the epidermis and may have a *sebaceous gland* opening into it.

   H.P. The follicle consists of parts corresponding to the dermis and epidermis, which are arranged as follows, travelling from the cutis vera towards the hair:—

   Epidermic covering. Dermic covering.
   - (*a*) *Outer sheath of the hair sac*; longitudinal closely packed fibres, running parallel to the hair.
   - (*b*) *Inner sheath of the hair sac*; transverse fibres, therefore cut across.
   - (*c*) *Hyaline layer*; clear layer separating dermic and epidermic portions.
   - (*a*) *Outer root sheath*; mass of irregular cells, continuous with the rete mucosum.
   - (*b*) *Inner root sheath*, corresponds to the horny layer. Consists of two sets of cells, viz., Henle's layer, of flat irregular non-nucleated cells, and Huxley's layer, of flat nucleated cells.
   - (*c*) *Cuticle of the root sheath*; a layer of imbricate cells in contact with those of the hair.
   - (*d*) *Hair*, with its various parts in section.

5. **Transverse section of hair follicle**; logwood, C. Balsam.

   Examine well under L.P. and H.P., comparing with the parts described above.

XVIII.—EYE.

I. Cornea.

1. **Vertical section**; logwood, C. Balsam.

L.P. The anterior deeply stained line is the *epithelium* continuous with that of the conjunctiva; the general body of the cornea is not well stained; the thin, well stained line is the *epithelium* of the anterior chamber.

H.P. Examine the characters of the various layers, passing from the exterior.

1. *Epithelium*; this is *stratified*, the inner cells being columnar, nucleated, and gradually passing to the flattened superficial ones.

2. *Anterior hyaline layer*, clear with no striations.

3. *Corneal substance*; formed by lamellæ of *fibrous tissue* placed at right angles in alternate layers, so appearing in longitudinal and transverse sections; between these layers are the well stained *corneal corpuscles*, appearing in rows.

4. *Membrane of Descemet or Demours* (posterior hyaline membrane) is clear and has a distinctly marked outline.

5. *Epithelium* of the anterior chamber, is formed of a *single* layer of *flat cells*, showing distinct *nuclei*.

2. Examine the gold preparation of the frog's cornea (page 14) for the *corneal corpuscles*. They are flat, angular in shape, *nucleated*, and throw off numerous branches.

II. Iris, etc.

1. **Junction of iris, sclerotic and cornea**, in section; logwood, C. Balsam.

L.P. Carefully note the relation of these parts.

The cornea at its junction with the sclerotic has its external epithelium continued as a finer band over the *conjunctiva*, which is of fine areolar tissue.

At the connection of the cornea and sclerotic with the iris, are seen the *spaces of Fontana*, bounded by the *ligamentum pectinatum iridis* from the anterior chamber.

Anterior to these spaces lies the *canal of Schlemm*, seen in section, while posterior and to the side is the *ciliary muscle*.

The iris is continuous with the choroid coat, showing the *ciliary processes* in section at their junction, and has a layer of *pigment cells*.

H.P. The sclerotic is composed of *fibrous* connective tissue, which becomes lighter towards the conjunctival portion. The character of the layers of the cornea may be well seen; also its lining of epithelial cells over the membrane of Descemet.

The light tissue of the ciliary processes may be distinguished, with their *single* layer of pigmented epithelium, the pigment being placed at the base of the cells which show a clear outline. Examine for the ciliary muscle of *unstriated* fibres.

The iris with its *pigmented* connective tissue is covered *anteriorly* with a single layer of cells continuous round the edge with the *posterior pigmented* layer, they are also continuous with that layer placed on the membrane of Descemet in the cornea. Towards the edge of the iris the *sphincter muscle* appears clearly; the unstriated fibres showing in transverse section with distinct nuclei. Throughout the section numerous *blood vessels* appear.

III. Lens.

1. Tease in glycerine a small piece of the lens treated for two or three days in $\frac{1}{8}$ % potassium bichromate.

   H.P. Notice the fibrous structure of this body, the *fibres* being long and with *serrated* edges.

2. Section; glycerine.

   H.P. The thin, flat fibres forming the substance of the lens, are seen to be placed in concentric lamellæ. When the capsule is present it appears as a clear, thin layer.

IV. Retina.

1. **Pig's retina,** section; logwood, C. Balsam.

   With H.P. note the following layers.

   1. *Nerve fibres*; form a layer lined inside by the *internal limiting membrane*. At different points sections of small *blood vessels* appear.

2. *Nerve cells*; forming a distinct layer, are *multipolar* and show clearly their nuclei and nucleoli.

3. *Inner molecular layer* has a granular appearance.

4. *Inner nuclear layer*; well stained *cells*, spindle-shaped with distinct nuclei, may be seen in several rows.

5. *Outer molecular layer*; thin layer, having a similar appearance to 3, not well stained.

6. *Outer nuclear layer*; like 4 is formed of well stained spindle shaped nucleated *cells*, in several rows.

7. *External limiting membrane*, appears as a clear line outside which lies—

8. *The layer of rods and cones*, which in this preparation are not distinct.

Stretching directly across this section from the internal limiting membrane *Müller's fibres*, with a clear outline, may be distinctly seen.

2. **Frog's retina**; prepared with osmic acid, tease in glycerine.

H.P. shows the *rods* distinctly, which break up into a long, clear, *outer* portion and a smaller, granular, *inner* portion.

3. **Pigment layer.** Given as a specimen of epithelium (page 12).

In some sections of retina the pigment layer appears; the cells throw numerous processes between the rods and cones, in which processes and the lower part of the cells the pigment granules collect, leaving a clear *outer* hyaline border.

XIX.—OLFACTORY MUCOUS MEMBRANE, etc.

I. Olfactory Mucous Membrane.

I. Guinea-pig, section; logwood, C. Balsam.

L.P. shows an *epithelial layer* resting upon a basement membrane, and covering fine *connective tissue* containing *glands* (of Bowman) and nerve fibres.

H.P. Epithelium is seen to consist of several layers; the upper cells are *columnar* with well stained nuclei; below these (more clearly seen where the epithelium is slightly broken up) are several layers of well nucleated *spindle-shaped cells*, sending up processes between the columnar ones.

The glands are *serous*, with distinctly nucleated cells.

2. Tease in glycerine a piece of the membrane from a guinea-pig, treated for 24 hours in Ranvier's alcohol, and stained with carmine. H.P. Study the above cells separated.

II. Tactile Corpuscles and Pacinian Bodies.

Examine the sections of skin (page 43).

Demonstrations.

1. Osmic acid preparations of skin to show the tactile corpuscles.

2. Silver preparations of Pacinian bodies to show the endothelial nature of their coats.

III. Taste Buds.

1. **Rabbit's tongue; papilla foliata**; logwood; C. Balsam.

L.P. The epithelium is in folds, covering a mass of striated muscle embedded in connective tissue. On each side of these crypts, well stained oval buds may be seen, *taste buds.*

H.P. Carefully examine the buds. The general epithelial cells flatten as they approach them. The buds show an *outer layer* of nucleated cells, containing a bundle of *taste cells* which are *spindle shaped* with oval nuclei. At points the spaces containing these cells will show a small *opening* into the crypt.

XX.—BRAIN AND SPINAL CORD.

I. Spinal Cord.

1. **Cervical region** of dog; logwood, clear in turpentine and kreosote; mount in C. Balsam.

L.P. The cord, surrounded by a layer of fine connective tissue the *pia mater*, is divided into right and left

halves by the broad, shallow *anterior fissure* (into which dips the pia mater) and the narrow, deep *posterior fissure.*

The outer portion of the cord is formed of *white substance*, which on each side consists of three divisions, viz., the *anterior, lateral* and *posterior columns* (the latter again being divided into two by a fine wedge of connective tissue called the slender fasciculus, peculiar to the cervical region).

The *grey matter* of the spinal cord has the shape of the letter H; each half of the cord possessing an *anterior* and a *posterior cornu*, joined across the median line with those of the opposite side, by the *grey commisure*; the latter shows in section the *central canal.* Each anterior cornu is broad and gives off its nerve fibres to the anterior root of the spinal nerve by *several* small bundles, while the posterior cornu is narrower and sends off its nerves by *one* compact mass: at this latter point the cornu has a light appearance, the *substantia gelatinosa.* The portion of the grey commisure behind the central canal is called the *posterior grey commisure*; that before it, the *anterior grey commisure*; while between the latter and the anterior fissure the *white commisure* is found, lightly stained.

H.P. The pia mater is of fine *connective tissue* containing numerous *blood vessels.*

The white matter is chiefly formed of *medullated nerve fibres*, seen principally in transverse section, with well stained *axis cylinders*; supporting these fibres is a very fine mass of (modified connective) tissue, *neuroglia*, cells of which may be seen stained, it is collected in a good layer just beneath the pia mater and also in the substantia gelatinosa.

The grey matter consists of medullated and non-medullated *nerve fibres* and *nerve cells*, held by neuroglia. The cells are *multipolar*, each having a distinct nucleus and nucleolus, and are larger in the anterior than in the posterior cornua. The central canal is lined by a layer of *columnar* epithelium.

2. **Dorsal region**; prepared from the same dog and mounted as before.

L.P. Compared with the cervical region, the section is smaller, and the quantity of white matter is smaller in proportion to the grey.

Study under both powers the parts described in the cervical region and note *Clarke's column*, a small collection of large cells, outside and posterior to the central canal.

3. **Lumbar region**, mounted by the same method.

Under both powers work as before; the proportion of white matter is still less compared with the grey; the cornua are large.

4. **Lumbar region of spinal cord of ox**; transverse section; aniline blue, glycerine.

Use L.P. and H.P. Note the fine *neuroglia*, which is very distinct in the white substance, with the numerous medullated nerve fibres having well stained axis cylinders. The *multipolar* nerve cells with their nuclei and nucleoli are very large in the anterior cornua.

II. Cerebellum.

1. **Vertical section**; logwood, C. Balsam.

L.P. Note the primary and secondary folds forming the *folia* or leaflets. *Grey matter* (outside) is covered by pia mater, and consists of:—

1. An outer, broad layer showing a few stained points.
2. An inner, well stained *granular* or *nuclear layer*.

The *white matter*, enclosed in a fold of grey matter, is formed of numerous medullated nerve fibres.

H.P. The outer, broad layer of the grey matter shows a matrix with numerous fibres, and at parts stained *nuclei*; of the inner, nuclear layer the *nuclei* are very numerous. At the line of junction of these two layers is a single row of large, multipolar nerve cells, the *ganglion cells of Purkinje*, each of which possesses a distinct nucleus and nucleolus, and sends a fine single branch inwards to disappear in the nuclear layer, and a branch outwards which breaks up into numerous filaments in the outer layer.

III. Cerebrum.

1. **Vertical section**; carmine, C. Balsam.

L.P. shows the folds to be simple, and to consist of an outer *grey* and inner *white* matter.

H.P. Grey matter contains numerous *multipolar nerve cells* with nuclei and nucleoli; these at times may be distinguished as forming several layers, differing according to the size and number of the cells.

XXI.—ORGANS OF REPRODUCTION.

I. Male.

1. **Testis** of dog. Make a section passing through the tubuli seminiferi, epididymis and vas deferens, near the globus major: logwood, mount in C. Balsam.

L.P. Note the visceral portion of the *tunica vaginalis*, within which lies the *tunica albuginea* throwing in branches to support the convoluted *tubuli seminiferi* and becoming thicker at the connection with the *epididymis*. The tubes of the latter are readily seen by their clear lumen and regular, nucleated cells. The *vas deferens* appears as a tube in transverse section, having a large lumen and surrounded by muscular fibres.

Under H.P. the tunica vaginalis has a clear outline (section of the endothelial cells lining the sac), it is built of fibrous tissue showing many nuclei, and is named the *tunica adnata*.

Within the above lies the *tunica albuginea* of fibrous tissue, sending in numerous branches of fine *connective tissue* between the tubuli seminiferi. Blood vessels are seen in section.

The tubuli seminiferi, cut in various directions, consist of several layers of *seminal cells*, granular, nucleated, and differing according to the state of the gland; those cells nearest the lumen may be in the act of forming spermatozoa, hence called *spermatoblasts*.

The epididymis shows numerous sections of *tubes* embedded in a good quantity of *connective tissue*. The tubes of the epididymis possess a distinct coat of *circular muscular fibres*, within this a *basement membrane* and a layer of nucleated *columnar epithelium cells* with long *cilia*. Beneath these columnar cells the nuclei of others, placed on the basement membrane, may be seen.

The vas deferens is well embedded in connective tissue showing numerous blood vessels; it possesses an *external longitudinal muscular layer* and an *internal circular muscular layer* of the unstriated variety. Upon a layer of connective tissue is placed one of *stratified columnar epithelium*, the columnar cells being towards the lumen.

2. **Tubuli seminiferi** of the rat. Stain the section with logwood, and mount in C. Balsam.

L.P. Sections of the tubules separate, the amount of connective tissue between them being very small.

H.P. Examine different sections for the *spermatozoa* in various stages of development. The seminiferous tubules have a *basement membrane* and several layers of well nucleated *seminal cells*; those cells at the base being closely packed, while those near the lumen, the *spermatoblasts*, vary in appearance. Some appear fan-like with numerous short, well-stained rods, the heads of the spermatozoa; other tubes have cells with fine radiating, thread-like lines passing from these stained heads into the lumen, and in some cases filling the latter. At parts the tubes may have been crushed and the *spermatozoa* set free, the latter showing a flat ellipsoidal *head*, a short *middle piece* and a long *tail*.

3. **Spermatozoa**; cut across the testis of a fresh-killed rat, press the cut part on a slide, cover and examine under H.P. Note the *head piece*, *middle piece*, and *tail*, also the rapid movement due to the motion of the latter.

II. Female.

1. **Ovary** of cat; logwood, C. Balsam.

L.P. The section is seen to have a clear outline except at its attachment; it consists of a *stroma* showing sections of blood vessels, *Graafian follicles* of various sizes, and possibly a *corpus luteum*.

H.P. The clear outline is due to a single layer of short, columnar epithelium cells, the *germinal epithelium*, beneath which lies a distinct layer of fibrous tissue, the *tunica albuginea*. The stroma is formed of fibrous tissue containing blood vessels, and passes gradually from the tunica albuginea.

The *Graafian follicles* are found of various sizes; the smallest form a layer beneath the tunica albuginea, staining less deeply than that coat. Those somewhat larger show a distinct outline, the *membrana propria*, within which lies a single layer of columnar epithelium, the *membrana granulosa*; the latter encloses the *ovum* with its nucleus or *germinal vesicle*.

The largest follicles have a good fibrous investment, within which lies the *membrana granulosa* now many cells deep and not filling the cavity but throwing in a projection, the *discus proligerus*, which encloses the *ovum*. The ovum has a distinct, investing membrane, the *zona pellucida*, and shows the *germinal vesicle* with a *germinal spot*.

Some specimens may contain a *corpus luteum*, which has a fibrous appearance.

2. Uterus of cat; transverse section, logwood, C. Balsam.

L.P. shows a *mucous coat* containing numerous tubules, and surrounded by an *internal circular muscular* coat and an *external longitudinal muscular* coat; sections of numerous blood vessels may be seen.

H.P. The surface of the mucous coat has a covering of (ciliated) *columnar epithelium* (which may not appear in the section). The *tubules* are formed of (ciliated) *columnar cells*, show a distinct lumen and take a somewhat irregular course towards the muscular coat, they will therefore appear at some points in transverse section.

Both muscular coats are formed of the *unstriated variety* of muscle fibres.

PREPARATION OF THE TISSUES, HARDENING AGENTS, ETC.

I.—HARDENING AGENTS.

The tissues to be hardened must be removed from the animal as soon after death as possible. They should be divided into pieces of about $\frac{1}{4}$ to $\frac{1}{2}$ inch in size unless otherwise directed, and if covered with much blood may be dipped into normal saline for its removal. The volume of the fluid into which they are placed should be about 20 to 30 times that of the tissue.

After hardening, and when required for preservation, they are placed in spirit. It is best to gradually increase the strength of the latter, placing the tissue for 12 hours in 40 %, for 24 hours in 60 %, and then transfering to ordinary alcohol.

The reagents are best kept of such a percentage that they may be diluted to the strength required for any particular specimen.

Chromic Acid 1 %. Weigh 10 grammes of crystallized chromic acid and dissolve in 1 litre of distilled water.

Chromic Acid and Spirit. This must be prepared *just* before use and allowed to cool. The usual mixture is made by adding equal volumes of ·5 % chromic acid and spirit. It requires frequent renewal during the hardening process.

Potassium Bichromate 2 %. Dissolve 20 grms. of potassium bichromate in 1 litre of water.

Ammonium Bichromate 5 %. Dissolve 50 grms. of ammonium bichromate in 1 litre of water.

Müller's fluid. Dissolve 25 grms. of potassium bichromate and 10 grms. of sodic sulphate in 1 litre of water.

Osmic Acid 1 %. Should be kept in a dark place or in a bottle surrounded by a coating of brown-paper.

Picric Acid. Place a good quantity of the crystals in water for several days, shaking the bottle now and again. It may be filtered from the undissolved crystals.

Ranvier's Alcohol (alcool au tiers). Mix 1 part of rectified spirit with 2 parts of distilled water.

II.—STAINING AND MOUNTING REAGENTS.

Hæmatoxylin (Kleinenberg's).

(*a*) Saturate some 70 % alcohol with crystallized calcium chloride by well shaking, allow it to stand; pour off the fluid and add excess of powdered alum, again shake and allow it to stand; filter.

(*b*) Saturate some 70 % alcohol with powdered alum, filter off the alcohol, and mix this with the first filtrate in the proportion of 8 to 1.

(*c*) Saturate a *small* quantity of absolute alcohol with hæmatoxylin (which is very soluble), and add drops of this solution to the mixture of (*a*) and (*b*) till a moderately dark purple is obtained.

The solution may be weakened by the addition of the mixture of (*a*) and (*b*) if required. Specimens must be worked from *alcohol* to this staining fluid.

Logwood (slightly modified form of Rutherford's method).

Weigh 20 grms. of logwood chips, add 200 cc. of water, and place in a flask. Let this stand near a fire, or over a bunsen, at a temperature of about 70° C. for 8 hours (it must not boil). Leave in the flask for 24 hours, and then filter. To the filtrate add about 250 cc. of ·75 % alum solution till a fine purple is obtained; finally add 30 to 50 cc. of spirit. The solution does not

keep very long, but gives good results; it must be filtered each time before use, and the specimens must be worked from *water* to the logwood solution and washed in the same before mounting.

Carmine (Frey).

Place ·3 grms. of carmine in 30 cc. of distilled water and add drops of dilute ammonia till the carmine just dissolves; if the liquid smells strongly of ammonia leave it exposed in a warm place that the excess of ammonia may pass off. Add 30 grms. of glycerine and 4 grms. of alcohol, and shake well. Keep in a well stoppered bottle, and dilute with water according to the strength required.

Alum carmine.

Boil 1 grm. of carmine and 5 of alum in 100 cc. of water for 20 minutes over a hot-water bath. When cold, filter and boil up the filtrate with a little carbolic acid.

Picro-carmine.

Add a saturated ammoniacal solution of carmine to a saturated solution of picric acid till a precipitate occurs. Evaporate to one-fifth of its volume, cool, filter, and evaporate the filtrate to dryness. Make a watery solution of the residue of about 4 % strength; this may be diluted as required.

Picric acid.

The saturated solution may be kept and diluted as required.

Magenta (Rutherford).

For ordinary tissues the solution is made by dissolving 1 decigramme of magenta crystals in 9 cc. of rectified spirit, and adding 213 cc. of water. It should be kept in a well-corked bottle.

Magenta for blood (Ferrier).

Dissolve 1 decigramme of magenta in 15 cc. of distilled water, then add 5 cc. of rectified spirit and 20 cc. of glycerine.

Eosin.

Dissolve ·1 grm. of eosin in 100 cc. of distilled water. Before mounting, the specimen may be placed, for a short time, in some water acidulated with a few drops of acetic acid.

Aniline Blue.

Dissolve 1 grm. of aniline blue in 100 cc. of water.

Canada Balsam.

Place the C. Balsam in an evaporating basin and heat it, at a temperature of about 70° C., from 24 to 48 hours in a warm chamber, to drive off all moisture. When cold, break it up and dissolve in benzole.

Glycerine.

May be used as pure glycerine or diluted with an equal part of water.

III.—EPITHELIUM.

Cast Skin of Newt or Frog. Clean the animal by washing well, then leave 24 to 48 hours in about half a pint of clean water. Remove the cast skin, pin it out on a piece of cork and place in spirit for a short time to harden.

Columnar Epithelium. Cut about 2 or 3 inches of the small intestine of rabbit, rat, or frog. Pass a stream of water *very* gently through, by means of a syringe, to remove digestion products; then do the same with Ranvier's alcohol (alcool au tiers—1 part rectified spirit to 2 of water). Now tie up the opposite end, inflate gently with the Ranvier's alcohol, tie again at the near end and leave in the alcohol for 24 hours. Cut open and place in picro-carmine to stain. Gently brush the surface for specimens.

Ciliated Epithelium. Carefully remove the trachea of a rabbit or rat and treat in the manner just described. There is no necessity to tie the ends so as to inflate the tube.

Pigment Epithelium of Retina is easily removed from specimens of eye prepared as described on page 64, retina.

Epithelium of Bladder. Treat the bladder of a rabbit or cat with Ranvier's alcohol as described above.

Cornea. Take a section of cornea of cat or rabbit, hardened in 1 % potassium bichromate (page 64).

IV.—CONNECTIVE TISSUE.

Ligamentum Nuchæ. Remove from the neck of an ox and keep in alcohol.

Tendo Achillis of dog or cat; keep a few days in alcohol before taking sections.

Tail of young Rat. Keep for a few days in alcohol.

Cornea of Frog. Place it from 3 to 5 minutes in filtered lemon juice, dip in ·75 % salt solution, then place for 20 to 30 minutes in 1 % gold chloride; then for 20 to 24 hours in 25 % formic acid, within a dark chamber.

Tadpole's Tail. Place tadpoles for 2 days in saturated picric acid; next day 30 % spirit; next day 50 % spirit, then keep in spirit.

Omentum. Spread a piece on some cork and keep it 24 hours in spirit before use.

Lymphatic Gland. Remove one from a dog; make several deep incisions with a sharp razor and place in absolute alcohol.

V.—CARTILAGE.

Articular Cartilage. Take a piece from the articular surface of a long bone softened in chromic acid (Bone, page 59).

Femur of Frog. Remove the femurs of a frog and clear them from muscle, cut them across and leave in saturated picric acid till soft. The acid should be renewed now and again.

Costal Cartilage of dog, cat, etc. Place in $\frac{1}{6}$ % chromic acid for 14 or 16 days, frequently renewing the fluid. Then in 30 %, 50 %, 60 %, and strong spirit.

Epiglottis of sheep; in picric acid for 2 days, then in 50 % spirit till the colour is removed; keep in spirit.

Arytenoid of sheep. Remove the cartilages with a small piece of the vocal cords attached and place in absolute alcohol. Picric acid may be used as for epiglottis, if preferred.

Ligamentum Teres, for fibro-cartilage. Cut a section from the head of the femur (dog) prepared as in Bone (page 59).

Intervertebral cartilage, from rabbit, cat, or dog. Remove several intervertebral discs, with a small piece of the bone attached; divide into four by vertical cuts.

(1) in ·05 % chromic acid for 7 days (often renew).
(2) „ ·5 % „ „ 15 „ (or till soft) and often renew the fluid.
(3) weak and then strong spirit.

VI.—BONE.

Long Bone. Remove from a dog, and clear well from muscle. Cut into small pieces.

(1) in ·05 % chromic acid for 7 days.
(2) in ·5 % chromic acid for about 12 days, till the bone is soft and cuts easily. The fluids are to be often renewed and shaken up.
(3) place in weak and then strong spirit.

Picric acid, in good quantity and often renewed, may be used till the bone softens; it is then placed in weak and strong spirit.

Flat Bone. The frontal bone of a sheep may be treated in a similar manner.

Young Bone. Remove from a young kitten and keep in picric acid till soft: then in alcohol.

VII.—MUSCLE.

Unstriated, for teasing; place some thin slices of unstriated muscle (*e.g.* from small intestine) 24 hours before use, in Ranvier's alcohol.

Frog's bladder. Open the abdomen and empty the frog's bladder by pressure, ligature the large intestine, distend the bladder by a syringe (through the anus) with Ranvier's alcohol, and suspend in the same for 24 hours. The epithelium can then be easily removed by brushing, and the specimen stained.

Muscular walls of Intestine. Cut off 2 or 3 inches of the small intestine of a dog or cat.

(1) Gently clean the inside by a stream of normal saline.

(2) Gently send in a stream of chromic acid and spirit mixture; tie one end, distend with the mixture and then tie the other. Leave for 12 to 20 hours in same mixture.

(3) Cut open the intestine and leave in renewed mixture for 4 days.

(4) Place in 30 % spirit for 1 day and then in strong spirit.

Section of striated muscle.

Place some pieces of muscle from the dog, in ·5 % chromic acid for 4 days. Renew the fluid at the first and third day. Place in weak then strong spirit.

Cardiac: sheep. Cut some thin slices in the direction of the fibres and place in ·02 % chromic acid for 24 hours.

VIII.—NERVOUS TISSUE.

Osmic acid preparation of medullated nerves. Take the sciatic of the frog, gently tear open the sheath and place in 1 % osmic acid for two, three, or four hours.

Sections. Carefully remove a large nerve, spread over a piece of flat cork and place in saturated picric acid for 2 days. Remove to dilute spirit till all the colour is removed; keep in strong spirit.

Posterior Ganglia of spinal nerves, dog or cat. Place 2 days in picric acid, then in weak spirit till colour removed; keep in strong spirit.

Or keep 6 days in $\frac{1}{4}$ % chromic acid renewing at the second day; then in spirit.

Spinal Cord of ox. Cut pieces from the lumbar region of about $\frac{1}{4}$ inch in length. Place in 1 % potassium bichromate for 3 or 4 days, renewing as required. Stain with carmine. This is to be prepared when required.

IX.—BLOOD VESSELS.

Blood vessels. Wash out the vessels of a cat or dog with a gentle stream of normal saline. Then pour in a stream of 1 % potassium bichromate. Keep in the fluid for 6 days, renewing now and again. Remove to weak, then strong spirit.

Pia mater. Carefully remove the pia mater from a small animal and place in $\frac{1}{6}$ % chromic acid for 2 days; then in weak and finally in strong spirit. The head of a lamb is good to work from, and the surface of the brain may be removed in thin slices—the pia mater attached—and then placed in the acid.

Endothelial cells, from the bladder of a frog. Tie a ligature round the upper part of both hind limbs. Carefully open the abdomen and place a small canula, pointing towards the hind limbs, in the lower part of the aorta. With a syringe very gently inject some normal saline, to remove the blood from the vessels, and then remove the normal saline by a stream of distilled water. Next inject a gentle stream of ·5 % silver nitrate, leave it in the vessels for 3 or 4 minutes, and again wash out with distilled water. Inflate the bladder (page 59) with water and suspend in ordinary alcohol exposed to the light, till it turns light brown. Specimens may be cut off and mounted in C. Balsam.

X.—ALIMENTARY CANAL.

Tongue, of cat, dog, etc., may be kept in alcohol to harden.

Salivary glands. Carefully remove the glands required, make a few incisions with a razor and place in absolute alcohol to harden.

Tonsils; harden in absolute alcohol. Or place for 7 days in $\frac{1}{6}$ % chromic acid and keep in spirit.

Œsophagus. Remove the œsophagus and wash with normal saline. Wash with a stream of the chromic acid

and spirit mixture; tie one end, slightly inflate and suspend in the same for 24 hours. Cut across and place in fresh mixture for 3 or 4 days. Place in dilute and then strong spirit.

Stomach. Remove pieces from the cardiac and pyloric ends and spread over a piece of cork, after washing. Place in absolute alcohol to harden.

Intestine (*small and large*) may be hardened in absolute alcohol, as in the case of the stomach.

Or the mixture of chromic acid and spirit may be used. Wash a piece of intestine 2 or 3 inches long, distend with the mixture, ligature and place in the same for 12 hours. Cut open and leave in same for 4 days.

XI.—LIVER.

Liver, from pig or dog. Remove a piece, and make several cuts with the razor at distances of half an inch, then place in a good quantity of 2 % potassic bichromate for 10 to 11 days, renewing on the second and fifth day. Wash, then place in weak and afterwards strong spirit.

XII.—LUNG.

Trachea of dog. Remove and gently irrigate the trachea. Distend with $\frac{1}{6}$ % chromic acid and suspend in the same for 2 days: cut into pieces and place in $\frac{1}{4}$ % for 7 days. Place in weak then strong spirit.

Lung of kitten. Carefully remove and distend the lung with $\frac{1}{4}$ % chromic acid and suspend in the same for 18 hours; transfer to some fresh solution and leave it for 3 weeks Cut into pieces and place successively in weak and then strong spirit.

Newt's lung. Distend with ·5 % gold chloride, or spread in the same, the lung of a freshly killed newt, for 30 minutes. Keep in glycerine till required.

XIII.—LYMPHATIC SYSTEM.

Lymphatic Glands. Remove the compound lymphatic gland of a dog, make an incision with a sharp razor and place in absolute alcohol.

Thymus gland. Make some sharp incisions, then place for 24 hours in 50 % alcohol, then in strong spirit.

XIV.—SPLEEN.

Remove the spleen from a cat, make several incisions and place for 4 days in 5 % ammonium bichromate. Place in weak and then in strong spirit.

XV.—THE DUCTLESS GLANDS.

Supra renal, make a deep incision and place in absolute alcohol.

It may also be hardened by placing it in 2 % potassium bichromate for 2 weeks if a large one (*e.g.* horse) be used.

Thyroid; make some incisions and then place for 24 hours in 50 % alcohol, afterwards in spirit.

XVI.—KIDNEY.

Kidney of dog or guinea-pig. Make several deep cuts, place in 2 % potassium bichromate for 2 or 3 weeks (according to size) changing after the first 24 hours, and as required. Keep in spirit.

Ureter of dog; distend and keep in 1 % potassium bichromate for 24 hours; renew and keep in the same for 5 days, then change to spirit.

Bladder. Squeeze out the urine, gently wash out once or twice with ·75 % sodium chloride; slightly distend with a mixture of ·5 % chromic acid and spirit (in equal parts) and suspend in the same for 12 hours. Place in a fresh mixture for 4 days, then remove to spirit.

XVII.—SKIN AND HAIR.

After removal these may be spread on cork and hardened in spirit. Or they may be placed in picric acid for 2 or 3 days and then removed to spirit; the latter should be renewed till the picric acid ceases to stain it.

The nail is best treated with picric acid.

XVIII.—EYE.

Cornea and junction of *Cornea Iris and Sclerotic.* Carefully remove the eye of a cat, divide into an anterior and a posterior half. Carefully remove the crystalline lens from the anterior portion; cut the latter into two parts and place for 24 hours in 1 % potassium bichromate; renew this and leave for 14 days. Place in spirit.

Lens. Remove the eye of a sheep, divide into an anterior and a posterior half, cut carefully through the cornea and remove a piece, then place the remainder in Müller's fluid for 3 weeks, renewing as required. Keep in spirit. This is for sections.

Retina. Divide the eye of a pig into an anterior and posterior half; place the latter in a mixture of equal parts chromic acid ·25 % and spirit for 24 hours; renew and leave in the same for 7 or 8 days. Place in weak spirit and then in strong spirit.

Frog's retina. Place the posterior half of a frog's eye for 3 or 4 hours, before required, in 1 % osmic acid.

XIX.—TASTE BUDS, etc.

Olfactory mucous membrane of guinea-pig. Remove the nasal septum and place in $\frac{1}{6}$ % chromic acid for 7 days, changing as required. Keep in spirit.

Taste Buds. Remove the papillæ foliata from the back part of a rabbit's tongue and place in absolute alcohol. Sections to be made transversely to the lines.

XX.—BRAIN AND SPINAL CORD.

Spinal cord of dog. Carefully remove the cord and cut nearly through at points about ½ inch apart. Place for 2 weeks in 5 % ammonium bichromate, changing as required. Keep in weak spirit 2 days, then in strong.

Spinal cord of the ox. Divide longitudinally and transversely the lumbar region, into pieces of ½ inch in length, and place in 5 % ammonium bichromate for 16 to 20 days, renewing the fluid as required. Keep in spirit.

Cerebrum and Cerebellum. Remove carefully and cut in thin slices, perpendicular to the surface. Place in 2·5 % ammonium bichromate for 5 or 6 days; then in weak spirit. Keep in strong spirit.

XXI.—REPRODUCTIVE ORGANS.

Testis of dog. Divide and place in 5 % ammonium bichromate for 8 to 10 days according to size, then transfer to weak spirit for 24 hours, and keep in strong spirit.

Testis of rat. Place in picric acid (saturated) for 2 days. Keep in spirit.

Ovary of cat. Make a deep incision and place in an equal mixture of ·5 % chromic acid and spirit for 7 days, changing as required; then keep in spirit.

Uterus of cat. Very carefully inflate with the mixture of ·5 % chromic acid and spirit, and place for 24 hours in the same. Renew the fluid and after 3 days remove to weak, then strong spirit.